From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: FW: Transmission studies
Date: Friday, July 31, 2020 2:20:00 PM
Attachments: Spike-614 BW.pptx

Dear [redacted],

[redacted] wants an update on the S-614 studies (see below). Here is what I plan to tell him; I hope it is okay with you. I will let you know the virus titers as soon as I get them:

We infected hamsters with 1000 pfu of SARS-CoV-2 strains that differ only at position S-614, generated by [redacted] based on a Seattle isolate, and sacrificed them on days 3 and 6.

The body weight changes of the animals at 3 and 6 days after infection are shown in the attached slide.

Animals infected with the virus bearing S-614G appear to have lost more weight. Titration of lung and nasal turbinate samples from each group on Days 3 and 6 was performed yesterday; we will have the titer results on Monday.

Best,
[redacted]

From: [redacted]
Sent: Saturday, August 1, 2020 1:38 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Hi [redacted]

I hate to bother you (and apologize if you've already gotten this question from others), but I was wondering if you have an estimate of when you think studies of the D614G mutant in hamsters may be complete?

Thanks,
[redacted]

From: [redacted]
Sent: Wednesday, June 24, 2020 7:06 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Dear [redacted]

[redacted] has generated isogenic recombinant SARS-CoV-2 viruses (S-D614 and S-G614) based on the Seattle isolate and is sending them to us. We will be testing them in hamsters once we get them.

Best,
[redacted]

[redacted]

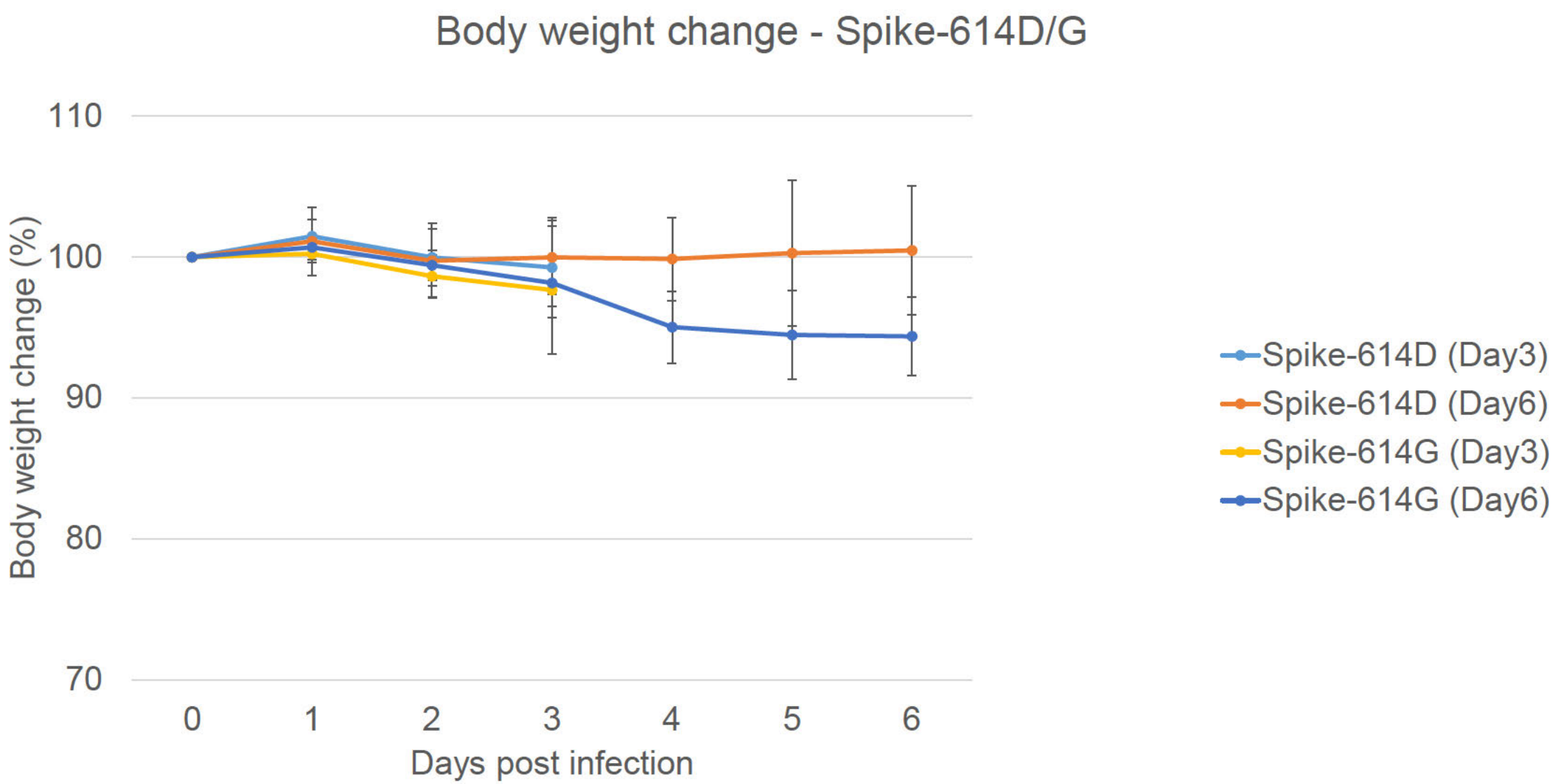
From: [redacted]
Sent: Tuesday, June 23, 2020 11:43 PM
To: [redacted]>
Subject: Transmission studies

Dear [redacted]

I apologize if I've already asked you this before, but are you planning any transmission studies that will compare the D614G spike variant with Wuhan-like isolates containing D614? I'm asking because there are a lot of questions following the recent Scripps study suggesting that the D614G increases infectivity in vitro.

Thanks,
[redacted]

[redacted]


Body weight change - Spike-614D/G
110
100
90
80
70
Body weight change (%)
0
1
2
3
4
5
6
Days post infection
Spike-614D (Day3)
Spike-614D (Day6)
Spike-614G (Day3)
Spike-614G (Day6)

From: [redacted]
To: [redacted] with [redacted]
Date: Friday, June 19, 2020 6:28:00 AM

[redacted]
We can use this opportunity to discuss our experiments with your mutants in hamsters.

[redacted],
Please join.

[redacted]

From: [redacted]
Sent: Friday, June 19, 2020 7:47 PM
To: [redacted]
Cc: [redacted]
Subject: About a meeting with [redacted]

[redacted]

This is something different from the interview we have scheduled.

Actually [redacted] also wants to have a little meeting with [redacted].
He said [redacted] from Madison is also joining.
Is the following time still available?

Tuesday, June 23 @ 8-9 am EST (June 23 @ 9-10 pm JST)

[redacted] is going to follow up with the topics he wants to discuss about.
Please let us know!

Thanks,
[redacted]

2020/06/15 21:42、[redacted]:

Dear [redacted]
That is so much better for [redacted]. Thank you!! His availability next week is:
6/23 Tuesday 8-9, 10-11 (Eastern time)
Wednesday 8-10 after noon
Thursday 11:30-4:00
Friday 8-10, 11-12

Please let me know if any of these times work.
Best regards,
[redacted]

From: [redacted]
Sent: Monday, June 15, 2020 7:43 AM
To: [redacted]
Cc: [redacted]
Subject: Re: Interview with a Japanese TV crew

Dear [redacted]

Thank you for your reply.
Actually, I was thinking sometime next week from the 22nd to 26th would be great for the interview.
your late evening or early morning would work perfect for us (I believe we're 13 hrs ahead).

I'd appreciate it if you could give us about 1hour of your precious time.
I will send you a list of questions we'd like to ask beforehand.

Thank you,
[redacted]

2020/06/15 3:09[redacted]

Dear [redacted]
[redacted] calendar is pretty full this week. He has some time on Tuesday evening and Thursday evening. Is there a time zone issue and would mornings be better? If so, Friday morning before 10:30 ET would work.
Best regards,
[redacted]

From: [redacted]
Sent: Sunday, June 14, 2020 12:16 PM
To: [redacted]
Cc: [redacted]
Subject: Re: Interview with a Japanese TV crew

Hello [redacted],

Thank you so much for accepting our offer for an interview.
My name is [redacted] a TV director working for NHK (Japanese Public Broadcasting Channel).

Currently, we're making a documentary program on how scientists have been working on viruses before and after this pandemic occurred,
and how they are moving forward on develop treatments for COVID-19.

[redacted] and his team will be the main cast for this program,
and we would also like to cover scientists overseas, who are working at the front line of SARS-CoV-2 studies.

[redacted] told us about [redacted], and suggested that it is important to let Japanese audience know about what you have been working on, devoting decades laying the groundwork for COVID-19 treatments in the states.
It is truly our honor to introduce you in this show.

It'd be great if we could have a remote interview sometime next week. (Using zoom or Skype)
And please let us exchange some emails beforehand
because I'd like to ask about the ongoing projects that you're working on right now.
For now, I'm trying to catch up reading your papers and journals.

Please let me know if you have any concerns or thoughts about this.

Thank you,
[redacted]

2020/06/14 21:26、[redacted]:

Hi [redacted]
[redacted] said be would be delighted.
[redacted]

Sent from Outlook Mobile

From: [redacted]>
Sent: Sunday, June 14, 2020 7:44:12 AM
To: [redacted]
Cc: [redacted]
[redacted];[redacted]
[redacted]
Subject: Interview with a Japanese TV crew

Dear [redacted]

I am writing to see if you have time to do a short interview with a Japanese TV crew. They are currently shooting the COVID-19 activities in my lab and they want to interview an expert on coronaviruses.

Please let me know if you have time. It would be nice to see you on Japanese TVE!

Best,
[redacted]

From: [redacted]
To: [redacted]
Subject: RE: About a meeting with [redacted]
Date: Monday, June 22, 2020 4:59:53 AM

I have updated the calling information on my end.

From: [redacted]
Sent: Monday, June 22, 2020 4:51 AM
To: [redacted]
Cc: [redacted]
Subject: Re: About a meeting with [redacted]

Dear all,

About the meeting tomorrow,
Can we use the link below instead?
Sorry about the confusion. Let me know if you have any problem.

Thanks,

[redacted] is inviting you to a scheduled Zoom meeting.

Topic: Zoom Meeting with [redacted]
Time: Jun 23, 2020 09:00 PM Tokyo

June 23, 2020, 8am ET

Join Zoom Meeting
[redacted]

Meeting ID: [redacted]
Password: [redacted]

One tap mobile
[redacted] US (Chicago)
[redacted] US (Houston)

Dial by your location
[redacted] US (Chicago)
US (Houston)
US (New York)
US (San Jose)
US (Tacoma)
US (Germantown)

Meeting ID: [redacted]
Password: [redacted]
Find your local number: [redacted]

2020/06/20 5:40、[redacted]

Thanks, [redacted]

Please see below.

[redacted]

[redacted]

トピック: With [redacted]
時間: 2020年6月23日 09:00 PM JAPAN
June 23, 2020, 8am ET

Zoom [redacted]

ミーティングID: [redacted]
パスワード: [redacted]サンノゼ)
[redacted](ニューヨーク)

所在地でダイアル
[redacted]サンノゼ)
ニューヨーク)
Tacoma)
Germantown)
シカゴ)
ヒューストン)
ミーティングID: [redacted]
パスワード: [redacted]
市内番号を検索: [redacted]

SIPで参加
[redacted]

H.323で参加
[redacted]国西部)
国東部)
ンド ムンバイ)
ンド ハイデラバード)
ヨーロッパ/中東/アフリカ)
オーストラリア)
港特別行政区)
ブラジル)
ナダ)
(日本)
パスワード: [redacted]
ミーティングID: [redacted]

From: [redacted]
Sent: Saturday, June 20, 2020 5:34 AM
To: [redacted]

[redacted]
Subject: RE: About a meeting with [redacted]

Hi [redacted]
It seems that all he does lately is Zoom. Yes he can. Copy me on the invite.

Hope you are doing well.
Best
[redacted]

From: [redacted]
Sent: Friday, June 19, 2020 4:31 PM
To: [redacted]
Subject: RE: About a meeting with [redacted]

[redacted]
Can [redacted] use Zoom?
Is so, I can send Zoom invitation.
[redacted]

From: [redacted]
Sent: Friday, June 19, 2020 11:48 PM
To: [redacted]
[redacted]
Subject: RE: About a meeting with [redacted]

Yes this time is still available. Should I use the same invitation that you sent before?
Best regards,
[redacted]

From: [redacted]
Sent: Friday, June 19, 2020 6:47 AM
To: [redacted]
[redacted]
Cc: [redacted]
Subject: About a meeting with [redacted]

[redacted]

This is something different from the interview we have scheduled.

Actually [redacted] also wants to have a little meeting with [redacted].
He said [redacted] from Madison is also joining.
Is the following time still available?

Tuesday, June 23 @ 8-9 am EST (June 23 @ 9-10 pm JST)

[redacted] is going to follow up with the topics he wants to discuss about.
Please let us know!

Thanks,
[redacted]

2020/06/15 21:42、[redacted]

Dear [redacted]
That is so much better for [redacted]. Thank you!! His availability next week is:

6/23 Tuesday 8-9, 10-11 (Eastern time)
Wednesday 8-10 after noon
Thursday 11:30-4:00
Friday 8-10, 11-12

Please let me know if any of these times work.
Best regards,
[redacted]

From: [redacted]
Sent: Monday, June 15, 2020 7:43 AM
To: [redacted]
Cc: [redacted]
Subject: Re: Interview with a Japanese TV crew

Dear [redacted]

Thank you for your reply.
Actually, I was thinking sometime next week from the 22nd to 26th would be great for the interview.
your late evening or early morning would work perfect for us (I believe we're 13 hrs ahead).

I'd appreciate it if you could give us about 1hour of your precious time.
I will send you a list of questions we'd like to ask beforehand.

Thank you,
[redacted]

2020/06/15 3:09、[redacted]

Dear [redacted]
[redacted] calendar is pretty full this week. He has some time on Tuesday evening and Thursday evening. Is there a time zone issue and would mornings be better? If so, Friday morning before 10:30 ET would work.
Best regards,
[redacted]

From: [redacted]
Sent: Sunday, June 14, 2020 12:16 PM
To: [redacted]
Cc: [redacted]
Subject: Re: Interview with a Japanese TV crew

Hello [redacted],

Thank you so much for accepting our offer for an interview.
My name is [redacted], a TV director working for NHK (Japanese Public Broadcasting Channel).

Currently, we're making a documentary program on how scientists have been working on viruses before and after this pandemic occurred,
and how they are moving forward on develop treatments for COVID-19.

[redacted] and his team will be the main cast for this program,
and we would also like to cover scientists overseas, who are working at the front line of SARS-CoV-2 studies.

[redacted] told us about [redacted] and suggested that it is important to let Japanese audience

know about
what you have been working on, devoting decades laying the groundwork for COVID-19 treatments in the states.
It is truly our honor to introduce you in this show.

It'd be great if we could have a remote interview sometime next week. (Using zoom or Skype)
And please let us exchange some emails beforehand
because I'd like to ask about the ongoing projects that you're working on right now.
For now, I'm trying to catch up reading your papers and journals.

Please let me know if you have any concerns or thoughts about this.

Thank you,
[redacted]

2020/06/14 21:26、[redacted]

Hi [redacted]
[redacted] said be would be delighted.
[redacted]

Sent from Outlook Mobile

From: [redacted]
Sent: Sunday, June 14, 2020 7:44:12 AM
To: [redacted] >
Cc: [redacted]
[redacted]
Subject: Interview with a Japanese TV crew

Dear [redacted]

I am writing to see if you have time to do a short interview with a Japanese TV crew. They are currently shooting the COVID-19 activities in my lab and they want to interview an expert on coronaviruses.

Please let me know if you have time. It would be nice to see you on Japanese TVE!

Best,
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Address for shipping virus
Date: Monday, July 6, 2020 4:33:00 PM

Thanks, [redacted]

[redacted]

From: [redacted]
Sent: Tuesday, July 7, 2020 6:26 AM
To: [redacted]
Cc: [redacted]
Subject: Re: Address for shipping virus

I'll get the virus sent out tomorrow morning.

[redacted]

On Jul 6, 2020, at 5:21 PM, [redacted] > wrote:

Please use Influenza Research Institute FedEx account [redacted]

From: [redacted]
Sent: Monday, July 6, 2020 4:16 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Address for shipping virus

Please send to

[redacted]

[redacted] – can you provide our FedEx acct #?

thanks

From: [redacted]>
Sent: Monday, July 6, 2020 3:56 PM
To: [redacted]
[redacted]>
Cc: [redacted]
Subject: RE: Address for shipping virus

Sorry. [redacted] please

From: [redacted]
Sent: Monday, July 6, 2020 10:21 PM
To: [redacted]
[redacted]
Cc: [redacted]>
Subject: RE: Address for shipping virus

[redacted], I don't believe you added [redacted] to the thread.

[redacted]

From: [redacted]>
Sent: Monday, July 6, 2020 9:16 AM
To: [redacted]
[redacted]
Cc: [redacted]
Subject: RE: Address for shipping virus

Great! Thanks, [redacted] and [redacted]!

[redacted],
Can you respond to [redacted]?
[redacted]

From: [redacted]
Sent: Monday, July 6, 2020 10:12 PM
To: [redacted]
[redacted]
Cc: [redacted]
Subject: Address for shipping virus

[redacted]

I'd like to get the virus shipped out today or tomorrow for you from the [redacted] lab. Could you please provide your shipping address, a phone number, and a FedEx account number if you have one? Thanks!

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] call agenda for tomorrow (Monday)
Date: Sunday, July 5, 2020 2:51:00 AM

Thanks, [redacted]

[redacted]

From: [redacted]
Sent: Sunday, July 5, 2020 4:37 PM
To: [redacted]
Cc: [redacted]
Subject: [redacted] call agenda for tomorrow (Monday)

[redacted]

Re the agenda for our meeting tomorrow. After general updates that will likely not take much time, I suggest we get into some planning discussions. We have been working on reanalyzing existing data, we could give an update on that and from there go into a discussion on planning. A rough outline of possibilities below, it will make more sense with discussion (we hope).

[redacted]

All [redacted] for a subset of these viruses:

- a [redacted] maybe the cell version of the current vaccine

- a [redacted]

- a [redacted]

We had also been thinking of some other experiments to add to the discussion of what our priorities should be:

- [redacted]

- [redacted] experiments. For example, [redacted] (early, middle, and lates in other lineages), and test against human sera that we select from the

human-sera experiment on that cluster.

- [redacted], looking at the [redacted].

- The pilot we have all discussed of [redacted] results due soon).

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] call on Monday
Date: Friday, May 29, 2020 12:42:00 PM

I am fine to postpone.

[redacted]

From: [redacted]
Sent: Saturday, May 30, 2020 12:02 AM
To: [redacted]
[redacted]>
Cc: [redacted]
Subject: Re: [redacted] call on Monday

Monday is a bank holiday for me. Happy to postpone.
[redacted]

From: [redacted]
Sent: Friday, May 29, 2020 5:00 PM
To: [redacted]
Cc: [redacted]
Subject: [redacted] call on Monday

Our monthly partners call is scheduled for this Monday at the usual time.

We have some work to present, but it is not urgent. We are still in the middle of it, so on one hand it would go faster if we presented it a bit later when we have more results, on the other hand it would be great to get your thoughts now.

[redacted], if you are still very busy with corona work, perhaps you'd prefer to not have the call?

[redacted] perhaps you will still be away?

[redacted] you have already heard what we will present.

Please let me know whether you prefer to have our call on usual Monday.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters
Date: Wednesday, June 17, 2020 10:19:31 AM

Thanks [redacted] I just submitted the export request so our vet staff will be in touch.
[redacted]

From: [redacted]
Sent: Tuesday, June 16, 2020 9:47 PM
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters

[redacted] - here you go:

Institute: University of Wisconsin – Madison
PI: [redacted]
Contact email: [redacted]
Vet: [redacted]
Transport coordinator: [redacted]
Email: [redacted]
Phone #: [redacted]
n/a
Ship to:
[redacted]

From: [redacted]
Sent: Tuesday, June 16, 2020 7:39 PM
To: [redacted]
Cc: [redacted]>
Subject: Re: CoV-2 - mutant viruses in hamsters

Hi all,
To arrange the hACE2 export I need to submit a request through our online system. A screenshot of the questions is in the attached powerpoint slide. We have 1 male homozygous ko, 2 male and 4 female heterozygotes to send.
thanks,

[redacted]

[redacted]

From: [redacted]
Sent: Tuesday, June 16, 2020 7:57 AM
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters

Thanks, [redacted]

From: [redacted]
Sent: Tuesday, June 16, 2020 8:48 PM
To: [redacted]
Cc: [redacted]>
Subject: RE: CoV-2 - mutant viruses in hamsters

Hi [redacted], Can you get in contact with [redacted] regarding the hACE2 mice. [redacted] can help set up a zoom call. [redacted]

From: [redacted]
Sent: Monday, June 15, 2020 8:13 PM
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters

[redacted],

I have not yet heard from [redacted] about the shipment of hACE2 mice.

Let's have a call to discuss hamster experiments. Actually, can we have this discussion as a web discussion (e.g., Zoom)?
The crew of the TV program that you kindly agreed to have an interview with are

interested in shooting a web conversation. They can edit out anything we do not want to disclose to the world.

[redacted] should also join this discussion.

Best,

[redacted]

P.S. [redacted] has a [redacted] going!

From: [redacted]>
Sent: Tuesday, June 16, 2020 1:50 AM
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters

Hi [redacted] hope your doing well. I hope that [redacted] has been in contact regarding shipments of hACE2 transgenic mice. [redacted] has made and is making additional interesting mutants. He has the 614 mutant already made in spike and is evaluating its phenotype on primary cells and a series of related experiments including stability, S protein content and glycan shield status, and in hACE2 transgenic mice. We think this mutant also needs to go into hamsters (compared to seattle recombinant virus control) and likely ferrets for transmission studies. Be glad to talk. [redacted]

From: [redacted]
Sent: Wednesday, June 3, 2020 9:16 PM
To: [redacted]
Cc: [redacted]
Subject: FW: CoV-2 - mutant viruses in hamsters

[redacted],

We have the necessary IBC approval to infect hamsters with recombinant, mutant CoV-2 viruses at Madison.

Please let me know if you are still interested in sending us your mutant viruses for this study.

Also, for [redacted] SARS-CoV-2, please email me the exact construct information.
We need to get an approval from the [redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters
Date: Tuesday, June 16, 2020 8:30:00 PM

Thanks, [redacted]

[redacted]

From: [redacted]
Sent: Wednesday, June 17, 2020 10:15 AM
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters

Hi [redacted], separate MTA from the institution to you. [redacted]

From: [redacted]
Sent: Tuesday, June 16, 2020 8:44 PM
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters

Thanks, [redacted]

[redacted]

Once we get enough animals in Madison, I want to send some to Japan.
What approval do I need to do this?

Thanks,

[redacted]

From: [redacted]
Sent: Wednesday, June 17, 2020 9:39 AM
To: [redacted]
Cc: [redacted]

[redacted]
Subject: Re: CoV-2 - mutant viruses in hamsters

Hi all,
To arrange the hACE2 export I need to submit a request through our online system. A screenshot of the questions is in the attached powerpoint slide. We have 1 male homozygous ko, 2 male and 4 female heterozygotes to send.
thanks,
[redacted]

[redacted]

From: [redacted]
Sent: Tuesday, June 16, 2020 7:57 AM
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters

Thanks, [redacted]
[redacted]

From: [redacted]
Sent: Tuesday, June 16, 2020 8:48 PM
To: [redacted]>
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters

Hi [redacted], Can you get in contact with [redacted] regarding the hACE2 mice. [redacted] can help set up a zoom call. [redacted]

From: [redacted]
Sent: Monday, June 15, 2020 8:13 PM
To: [redacted]
Cc: [redacted]>
Subject: RE: CoV-2 - mutant viruses in hamsters

[redacted]

I have not yet heard from [redacted] about the shipment of hACE2 mice.

Let's have a call to discuss hamster experiments. Actually, can we have this discussion as a web discussion (e.g., Zoom)?
The crew of the TV program that you kindly agreed to have an interview with are interested in shooting a web conversation. They can edit out anything we do not want to disclose to the world.

[redacted] should also join this discussion.

Best,
[redacted]
P.S. [redacted] has a [redacted] going!

From: [redacted]
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Subject: RE: CoV-2 - mutant viruses in hamsters

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From: [redacted]
Sent: Wednesday, June 3, 2020 9:16 PM
To: [redacted]
Cc: [redacted]
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[redacted]

We have the necessary IBC approval to infect hamsters with recombinant, mutant CoV-2 viruses at Madison.

Please let me know if you are still interested in sending us your mutant viruses for this study.

Also, for [redacted] please email me the exact construct information.
We need to get an approval from the [redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: CoV-2 - mutant viruses in hamsters
Date: Tuesday, June 16, 2020 8:06:12 PM

Thanks [redacted] – I will get back to you on those questions from the screen shot.

Best,
[redacted]

From: [redacted]
Sent: Tuesday, June 16, 2020 7:39 PM
To: [redacted]
Cc: [redacted]
Subject: Re: CoV-2 - mutant viruses in hamsters

Hi all,
To arrange the hACE2 export I need to submit a request through our online system. A screenshot of the questions is in the attached powerpoint slide. We have 1 male homozygous ko, 2 male and 4 female heterozygotes to send.
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Subject: RE: CoV-2 - mutant viruses in hamsters

Hi [redacted] hope your doing well. I hope that [redacted] has been in contact regarding shipments of hACE2 transgenic mice. [redacted] has made and is making additional interesting mutants. He has the 614 mutant already made in spike and is evaluating its phenotype on primary cells and a series of related experiments including stability, S protein content and glycan shield status, and in hACE2 transgenic

mice. We think this mutant also needs to go into hamsters (compared to seattle recombinant virus control) and likely ferrets for transmission studies. Be glad to talk. [redacted]

From: [redacted]>
Sent: Wednesday, June 3, 2020 9:16 PM
To: [redacted]
Cc: [redacted]
Subject: FW: CoV-2 - mutant viruses in hamsters

[redacted],

We have the necessary IBC approval to infect hamsters with recombinant, mutant CoV-2 viruses at Madison.

Please let me know if you are still interested in sending us your mutant viruses for this study.

Also, for [redacted] please email me the exact construct information.
We need to get an approval from the [redacted]

[redacted]

From: [redacted]
To: [redacted]
Subject: RE: Discussion re [redacted] s email
Date: Sunday, July 5, 2020 2:49:00 AM

I am fine with either.

[redacted]

From: [redacted]
Sent: Sunday, July 5, 2020 4:42 PM
To: [redacted]
Subject: Discussion re [redacted] email

Would also be good to discuss [redacted] reply below. I have a little extra information on the [redacted] collaboration from [redacted] that I can pass along. Would you prefer to do that in the partners call on Monday, or on a separate call? I'm good with either.

Note, I got an out-of-office email from [redacted], extended leave until September.

[redacted]

On Fri, Jul 3, 2020 at 1:07 AM [redacted] wrote:

> Dear [redacted],
>
> Thank you for your email below. My sincerest apologies for not getting back to you sooner. We are facing an unprecedented volume of emails and phone calls.
>
> I shared your email with [redacted] who is the COR for the [redacted] contracts. She felt it would be a good idea to go ahead and contact [redacted]. The PI is [redacted] The funding for [redacted] all comes from one "pot". Unless another agency like [redacted] would like to fund through us, there is no other way for the money to come from a different place. I think generating the 3-5 pages regarding next steps and then having a discussion between us and [redacted] is a great idea (and we can definitely include [redacted]).
>
> Please let me know if you have any additional questions or if there is any additional information I can provide.
>
> Thank you!
> [redacted]
>
> From: [redacted]
> Sent: Thursday, June 25, 2020 10:50 AM
> To: [redacted]
> Cc: [redacted]
> Subject: Post-discussion follow up

Dear [redacted]

Thank you again for our previous conversation. Also, we have recently received news from [redacted] that you have granted us the options we discussed to continue our work on our [redacted] for the final months of the [redacted] contract. Many thanks for this, it is very much appreciated.

As per your suggestion, we have investigated the [redacted] possibility. We have spoken informally with [redacted] about us being involved there. [redacted] would very much like to work with us, but also thinks our current collective budget is higher than they can handle with options, especially given their own agenda and plans.

Though we have not talked with the [redacted], that is potentially an interesting one for us to partner with for subsequent generations of our approach given the basic work at [redacted] with [redacted], and immunology expertise. If they like [redacted] already have concrete plans of their own however, we'd be in the same situation as with [redacted].

Is there a possibility for us to join a [redacted] and for the money for our options to come from elsewhere, and if so where?

Also, in discussion with [redacted] at [redacted] re our pandemic vaccine approach, he suggested we write 3 to 5 pages that describes our plan for both [redacted] going forward, and that you and [redacted] discuss this. We suggested it might be useful to include [redacted] in those discussions too given our shared mission to continue to integrate our progress incrementally into the vaccine strain selection process and the synergy with [redacted] thinking too. Does that seem like a good next stop from your perspective or would you prefer something different?

With best wishes
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Draft email to [redacted]
Date: Wednesday, June 24, 2020 4:03:00 PM

Yes. Sounds good to me too.

[redacted]

From: [redacted]
Sent: Thursday, June 25, 2020 1:20 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Draft email to [redacted]

Sounds good to me.

Thanks,

[redacted]

From: [redacted]
Sent: Wednesday, June 24, 2020 9:26 AM
To: [redacted] >
Cc: [redacted]
Subject: Draft email to [redacted]

[redacted]

Below draft email to [redacted] as per our emails post [redacted] discussion and suggestion with [redacted] is good with how that discussion has been summarized. Please let me know any edits you'd like to make to the email, or if you are OK with it, then I'll send to [redacted]

[redacted]

Dear [redacted]

We have recently received news from [redacted] that you have granted us the options we

discussed to continue our work on our [redacted] work for the final months of the [redacted] contract. Many thanks for this, it is very much appreciated.

As per your suggestion, we have investigated the [redacted] possibility. We have spoken informally with [redacted] about us being involved there. [redacted] would very much like to work with us, but also thinks our current collective budget is higher than they can handle with options, especially given their own agenda and plans.

Though we have not talked with the [redacted], that is potentially an interesting one for us to partner with for subsequent generations of our approach given the basic work at [redacted] with [redacted], and immunology expertise. If they like [redacted] already have concrete plans of their own however, we'd be in the same situation as with [redacted].

Is there a possibility for us to join a [redacted] and for the money for our options to come from elsewhere, and if so where?

Also, in discussion with [redacted] at [redacted] re our pandemic vaccine approach, he suggested we write 3 to 5 pages that describes our plan for both [redacted] going forward, and that you and [redacted] discuss this. We suggested it might be useful to include [redacted] in those discussions too given our shared mission to continue to integrate our progress incrementally into the vaccine strain selection process and the synergy with [redacted]s thinking too. Does that seem like a good next stop from your perspective or would you prefer something different?

With best wishes
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Draft proposal for [redacted] no cost extension
Date: Friday, July 17, 2020 2:07:04 AM
Attachments: image001.png
image002.png
image003.png

Looks good to me.

[redacted]

Yours sincerely,

[redacted]

[redacted]

From: [redacted]>
Sent: Friday, July 17, 2020 1:36 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Draft proposal for [redacted] no cost extension

Dear All,

Attached please find a few suggestions from Madison.

Best,

[redacted]

From: [redacted]
Sent: Thursday, July 16, 2020 5:08 PM
To: [redacted]
Cc: [redacted]
Subject: Draft proposal for [redacted] no cost extension

Hi [redacted],

We are sending you our draft no cost extension proposal for [redacted]

We have not heard back from [redacted] re expected requirements for this document. So we have developed this document based on the information that other funders stipulate for no cost extensions e.g. NIH, but noting of course

our unique relationship with the folks at [redacted]

Please let us know if you think we should make any edits or adjustments.

[redacted] - what are your thoughts on whether to mention you have been able to do some virus passaging? Specifically with regards to our para on labs being largely shut down, which of course supports our case for extension.

Also, if either of your labs feel anything else should be included for tasks done during lockdown or edits in this regard please let us know.

Many thanks

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Export from UNC ([redacted]) to U. of Wisconsin - Madison ([redacted] E#13192
Date: Wednesday, June 24, 2020 4:13:00 PM
Attachments: image001.png

[redacted]

Thank you very much!

[redacted]

From: [redacted]
Sent: Thursday, June 25, 2020 1:13 AM
To: [redacted]
Cc: [redacted]
[redacted]>
Subject: Re: Export from UNC [redacted]) to U. of Wisconsin - Madison ([redacted]) E#13192

Great, thank you for letting me know!

From: [redacted]>
Sent: Wednesday, June 24, 2020 11:16 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Export from UNC [redacted] to U. of Wisconsin - Madison [redacted] E#13192

Good Morning [redacted]

Just wanted to let you know the mice arrived this morning and are doing fine.

Thanks for your help
[redacted]

[redacted]

From: [redacted]
Sent: Monday, June 22, 2020 1:54 PM

To: [redacted]
Cc: [redacted]
Subject: Re: Export from UNC ([redacted]) to U. of Wisconsin - Madison [redacted] E#13192

Hey [redacted]

I sent all the info I have on the mice in a previous email, but it might have gotten lost in the shuffle so I'll provide it here.

See below for the information I have in regards to the mice. If you need anything more specific, [redacted] from the [redacted] lab (cc'd here) will be able to provide you with more insight.

Num of Males	Num of Females	Strain	DOB
2		hACE2 het	4/13/20
	5	hACE2 het	4/30/20
1		hACE2 homozygote	5/10/20

Let me know if you need anything else on my end!

Best,
[redacted]

From: [redacted]
Sent: Monday, June 22, 2020 2:26 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Export from UNC [redacted] to U. of Wisconsin - Madison [redacted]) E#13192

Hi [redacted]

Thanks for the update, I will let you know when they arrive. Do you have any information on the mice that were shipped?

Thanks
[redacted]

[redacted]

From: [redacted]
Sent: Monday, June 22, 2020 1:06 PM
To: [redacted]
Cc: [redacted]
[redacted]>
Subject: Re: Export from UNC [redacted] to U. of Wisconsin - Madison [redacted]) E#13192

Good Afternoon [redacted]

The mice have been picked up by World Courier and are on their way. I have been updated by World Courier that there will be a hold on this export, so delivery will happen by 2pm on the 24th. The mice will be kept in a temperature controlled warehouse until they are transported to your institution. Please let me know when the mice have arrived.

Let me know if you have any questions or concerns.

Best,
[redacted]

From: [redacted]
Sent: Friday, June 19, 2020 9:30 AM
To: [redacted]
Cc: [redacted]
[redacted]
Subject: Re: Export from UNC [redacted]) to U. of Wisconsin - Madison [redacted]) E#13192

Good Morning,

I just finalized the shipment of mice from the [redacted] lab at UNC to the [redacted] lab at your institution. The mice will be picked up at UNC on 06/22 for a 06/23 delivery. The HWB is 3006880 and job number is 0627. [redacted] lab, please have the crates ready by 10 am. There will be 1 large crate (divided) to house your mice that I will leave on the DRC transport cart. Please use the hydrogel I provide and place it on the floor in each compartment in which there are mice along with pelleted food. Once the mice are ready, please return them to the cart for

pick-up. If anyone has any questions or concerns, please feel free to contact me.

Best,

From:
Sent: Thursday, June 18, 2020 3:51 PM
To: >
Cc:
Subject: RE: Export from UNC to U. of Wisconsin - Madison E#13192

Hi

You can use World Courier or Validated Delivery. Please send me information on the strain, sex, age and number of mice, I need to do a transfer form on my end.
Thanks

Ship To:

[redacted]

From: [redacted]>
Sent: Thursday, June 18, 2020 1:53 PM
To: [redacted]>
Cc: [redacted]
Subject: Re: Export from UNC [redacted]) to U. of Wisconsin - Madison [redacted]) E#13192

Good Afternoon [redacted]

Can you confirm your shipping address and provide me with your World Courier or preferred courier and account number?

Best,
[redacted]

From: [redacted]>
Sent: Thursday, June 18, 2020 2:21 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Export from UNC [redacted]) to U. of Wisconsin - Madison ([redacted] E#13192

Good Afternoon [redacted]

[redacted] has approved the transfer of mice from the [redacted] lab to the [redacted] lab.
Please let me know what you need from me for the transfer.

Best Regards
[redacted]

[redacted]

From: [redacted]
Sent: Wednesday, June 17, 2020 1:12 PM
To: [redacted]
Cc: [redacted]
[redacted]>
Subject: Export from UNC [redacted]) to U. of Wisconsin - Madison [redacted]) E#13192

Good Afternoon,

I have received an export request form from the [redacted] lab here at UNC. They are requesting to ship mice to the [redacted] lab at your institution. I have attached our health surveillance description along with the past years' worth of health reports from the [redacted] We will need a written approval to ship the mice before we can continue the export process on our end. Please feel free to contact me if you have any questions or concerns.

****Please reference E#13192 in the subject line when reference this export request.****

Best,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: FW: Re: FW: MSN238613 UNC In-MTA
Date: Thursday, July 16, 2020 7:20:00 AM

Thank you, [redacted]!

[redacted]

-----Original Message-----
From: [redacted]
Sent: Thursday, July 16, 2020 9:19 PM
To: [redacted]
Cc: [redacted]
Subject: RE: FW: Re: FW: MSN238613 UNC In-MTA

Hi [redacted],

Please find attached a copy of the fully executed agreement for your records. Let us know if we can provide any further assistance.

Thanks!
[redacted]

[redacted]

[redacted]

-----Original Message-----
From: [redacted]>
Sent: Tuesday, July 14, 2020 9:56 AM
To: [redacted]
Cc: [redacted]
Subject: FW: FW: Re: FW: MSN238613 UNC In-MTA

Dear [redacted]

Please see attached.

Best,

[redacted]

> -----Original Message-----

> From: [redacted]
> Sent: Tuesday, July 7, 2020 2:16 AM
> To [redacted]
> Cc: [redacted]
> Subject: RE: Re: FW: MSN238613 UNC In-MTA
>
> [redacted],
>
> Thanks for your email. I have accepted all of the proposed changes. The only thing that needs to be added is The University of Tokyo's legal address where I indicated on page 1. Please insert the legal address and feel free to initiate signatures. Let me know if there are any questions.
>
> Thanks,
> [redacted]
>
> [redacted]
> [redacted]
>
> [redacted],
> [redacted]
> [redacted]
> [redacted]
> [redacted]
> [redacted]
> [redacted]
> [redacted]
>
> -----Original Message-----
> From: [redacted]
> Sent: Sunday, July 5, 2020 9:54 PM
> To: [redacted]
> Cc: [redacted]
> Subject: FW: Re: FW: MSN238613 UNC In-MTA
>
> Dear [redacted]
>
> The U of Tokyo IP office wants to have some changes.
> Would you check?
>
> Thanks,
>
> [redacted]
>
> [redacted]
> [redacted]
> [redacted]
> [redacted]
> [redacted]
> [redacted]
> [redacted]
> [redacted]
>
>
>
>>> From: [redacted]
>>> Sent: Friday, June 19, 2020 4:19 AM

>>> To: [redacted]
>>> Cc: [redacted]
>>> Subject: RE: MSN238613 UNC In-MTA
>>>
>>> Dear [redacted],
>>>
>>> I attached the incorrect draft to the previous email. Please find attached the correct draft for your review.
>>>
>>> Best,
>>> [redacted]
>>>
>>> [redacted]
>>> [redacted]
>>>
>>> [redacted],
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]>
>>> [redacted]
>>> [redacted]
>>>
>>> From: [redacted]
>>> Sent: Thursday, June 18, 2020 2:37 PM
>>> To: [redacted]
>>> Cc: [redacted]
>>> Subject: FW: MSN238613 UNC In-MTA
>>>
>>> Dear [redacted],
>>>
>>> Please find attached a draft MTA for your review. Let us know if there are any questions or comments. If acceptable, please return a signed PDF to [redacted]>.
>>>
>>> Best,
>>> [redacted]
>>>
>>> [redacted]
>>> [redacted]
>>>
>>> [redacted],
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>>
>>> From: [redacted]
>>> Sent: Wednesday, June 17, 2020 3:43 PM
>>> To: [redacted]
>>> Cc: [redacted]

>>> <[redacted]>
>>> Subject: FW: MSN238613 UNC In-MTA
>>>
>>> Hello,
>>> Upon further conversations with [redacted], could [redacted] also have an agreement for his laboratory at the University of Tokyo? If you could send a draft agreement directly to [redacted] (cc'd), it would be greatly appreciated. Same material(s) as in the attached agreement, just for [redacted] through the University of Tokyo.
>>>
>>> Thank you,
>>> [redacted]
>>>
>>>
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>>
>>> [redacted]
>>> [redacted]
>>>
>>> This transmission may contain confidential or privileged information. Use of this information by anyone other than the intended recipient is not authorized and may be unlawful. If you receive this in error, please inform the sender and remove any record of this message. Transmissions cannot be guaranteed to be secure or error-free as information could be intercepted, arrive late, or be incomplete. Neither the sender nor the University of Wisconsin-Madison accepts liability for any errors or omissions in content that arise as a result of this transmission. E-mail communications with the University of Wisconsin may be considered public information.
>>>
>>>
>>>
>>> From: [redacted]
>>> Sent: Tuesday, February 18, 2020 1:34 PM
>>> To: [redacted]
>>> <[redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> Cc: [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> Subject: RE: MSN238613 UNC In-MTA
>>>
>>> Hi [redacted],
>>>
>>> Thanks for your email. Please find attached a copy of the fully executed agreement for your records. Let us know if we can provide any further assistance.
>>>
>>> Best,
>>> [redacted]
>>>
>>> [redacted]
>>> [redacted]
>>>
>>> [redacted],
>>> [redacted]

>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>>
>>> From: [redacted]
>>> <[redacted].
>>> [redacted]
>>> [redacted]
>>> [redacted] >>
>>> Sent: Thursday, February 13, 2020 5:05 PM
>>> To: [redacted]
>>> Cc: [redacted]
>>> Subject: MSN238613 UNC In-MTA
>>>
>>> Hello,
>>>
>>> I am contacting you from the Office of Research and Sponsored at the University of Wisconsin-Madison to let you know the above-referenced agreement has been partially-executed, and is attached here. Once it has been fully-executed please send me a copy for our records.
>>>
>>> Do not hesitate to contact me with any questions or concerns.
>>>
>>> Thank you!
>>>
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>> [redacted]
>>>
>

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Interview with a Japanese TV crew
Date: Sunday, June 14, 2020 7:28:00 AM

Terrific!
[redacted] will be contacting you shortly.

[redacted]

From: [redacted]
Sent: Sunday, June 14, 2020 9:27 PM
To: [redacted]
Cc: [redacted]
Subject: Re: Interview with a Japanese TV crew

Hi [redacted]
[redacted] said be would be delighted.
[redacted]

Sent from Outlook Mobile

From: [redacted]
Sent: Sunday, June 14, 2020 7:44:12 AM
To: [redacted]
Cc: [redacted]>; [redacted]
Subject: Interview with a Japanese TV crew

Dear [redacted]

I am writing to see if you have time to do a short interview with a Japanese TV crew. They are currently shooting the COVID-19 activities in my lab and they want to interview an expert on coronaviruses.

Please let me know if you have time. It would be nice to see you on Japanese TVE!

Best,
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] call debrief
Date: Thursday, June 4, 2020 5:49:00 PM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png
image007.png
image008.png
image009.png

I also agree with the plan.

[redacted]

From: [redacted]
Sent: Friday, June 5, 2020 4:30 AM
To: [redacted] >
Cc: [redacted]
Subject: Re: [redacted] e call debrief

Yes I agree with the plan. I think that if you send an email for a first contact, then they can indeed think about it and we can schedule a call later.

From: '[redacted]
Date: Thursday, 4 June 2020 at 20:56
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call debrief

i hear that [redacted], and it makes sense. especially as we don't know the [redacted].

do others concur? and if so, what are your thoughts re me, or all of us talking with [redacted]? or an email to them. i think me sending an email to them, with us copied, would be the better than a call, they just need the info, then they can have a think, and maybe a call after they've discussed internally. what do others think?

[redacted] how much of what you discussed with [redacted] do you think it is ok to put in an email?

[redacted]

On Thu, Jun 4, 2020 at 4:22 PM [redacted] wrote:

> I would have a preference to work with [redacted] so would prefer to talk with [redacted] first about extra funds. If they suggest we should go strengthen the [redacted] team and help them spend their money wisely, than I would reconsider
>
> [redacted]
>
> Yours sincerely,
>
> [redacted]

[redacted]

[redacted]:
[redacted]:
[redacted]:
CC: [redacted]
[redacted]: Re: [redacted] call debrief

Thanks for all this [redacted]. Very helpful.

I add a little extra info re [redacted]. [redacted] as you know is doing a [redacted]. [redacted] you had been helpful when [redacted] was looking around re who might be possible at [redacted]. After trying a couple of labs to see how flexible they would be with the arrangement with us, she settled on one that non of us had identified previously, the lab of [redacted]. He is one of the few [redacted] consortium, perhaps I mentioned that when we had our call.

[redacted] recently extended previous ideas she'd had and come up with an [redacted] That would require making constructs that [redacted]. She presented that at our lab meeting on Tuesday, and [redacted] said that that is right in the research area of the [redacted] lab, that the [redacted] lab is currently pathogen-agnostic, but working on basic structural methods, and is very open to collaboration, and through [redacted] knows of our work (not anything that is not already public knowledge).

In a follow up call with [redacted] she also thinks that the [redacted] consortium [redacted].

What do people think as to whether it is worth a call with the [redacted] people, or [redacted] first, before we go back to [redacted]?

[redacted]

On Thu, Jun 4, 2020 at 3:52 PM [redacted] > wrote:

> I spoke briefly with [redacted] about [redacted]
>
> In general, he said that [redacted] are a bit more milestone driven than the [redacted] Options are both internal and external (so competitive if we come in). [redacted] rank the options together. The three [redacted] are similar in size (funding) and have similar options.
>
> [redacted] would very much like to work with us, but also thinks our current collective budget is higher than they can handle with options, as we already knew. If [redacted] bring in EXTRA money, than that could work for him [redacted] is [redacted] so [redacted] would need to discuss with him also, but doesn't see a problem himself.
>
> [redacted] also said that budgets for GMP production and clinical trials would NOT come from the [redacted], and would thus NOT be competitive with their own work. NIH would judge which candidates need to go into production and into clinical trials and pay for it. The sample processing from clinical studies could still go as (relatively smaller) options, e.g. via [redacted]. So, if we could do most of our work via clinical study, and smaller parts via [redacted]) that might work. Still, preferable to bring in extra money as anything we ask for would compete with [redacted] and [redacted]

[redacted] thinks that [redacted]. They have [redacted]. One is on [redacted]. In theory, they could be interested in our concept as they have [redacted]. But [redacted] would very much like to bring us into [redacted] if we do not take too much of their money.

So with that, I think [redacted] can have another call with [redacted] and [redacted] and separately with [redacted]?
We have a place to "land", but the pockets of [redacted] are not deep enough. Any possibility of routing extra money into Mt [redacted]?
BTW [redacted] said that some organization (NCI?) has routed COVID funds into the [redacted] so it can be done....

Yours sincerely,

[redacted]

[redacted]

[redacted]
[redacted]
[redacted]
CC: [redacted]
[redacted]
[redacted]**:** Re: [redacted] call debrief

Then let's not do today.

Guessing the same time constrains given our timezones, please reply with whether you could do the same times Mon Tue or Wed next week.

6 or 7am CT
1 or 2 pm NL
8 or 9pm Tokyo

[redacted]

On Fri, May 22, 2020 at 9:14 AM [redacted] wrote:

> I will indeed not be able to join. I will be out of the office the coming week.
> Best
> [redacted]

From: [redacted]
Sent: Friday, May 22, 2020 9:44:20 AM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call debrief

I will try to connect. [redacted] will – most likely - not.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
[redacted]: [redacted]
[redacted]
CC: [redacted]
[redacted]: Re: [redacted] call debrief

[redacted],

Could you do a 30 minute (I estimate) call at 13h or 14h your time today? Or suggest another day/time that works for you please.

The list of CIVICs here https://www.niaid.nih.gov/research/civics

[redacted]

On Thu, May 21, 2020 at 10:58 PM [redacted] > wrote:

> I am available after 6-8 am CT on May 22.
>
> [redacted]
>
> ---
>
> **From:** [redacted]
> **Sent:** Thursday, May 21, 2020 11:14 PM

To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] call debrief

Any time after 5 am CT will be okay.
There is a CEIRS Webinar at 9:30 am CT, but I can skip it.

[redacted]

From: [redacted]
Sent: Thursday, May 21, 2020 9:10 AM
To: [redacted]
Cc: [redacted]
Subject: [redacted] call debrief

I suggest we have a zoom debrief from this call tomorrow.

To discuss which center to approach, and how to approach.

Please email when you could do a 30 minute Zoom tomorrow so we can set a time. I can make any time work.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: New [redacted] options
Date: Wednesday, June 24, 2020 6:13:00 AM

Great. We can work on flu.

[redacted]

From: [redacted]
Sent: Wednesday, June 24, 2020 7:32 PM
To: [redacted]
Cc: [redacted]
Subject: New [redacted] options

We have been granted new [redacted] options from NIH.

The total amount we asked for after the catch up with [redacted] about 8 months ago. Perhaps this got actioned after our call with [redacted] last month.

You might remember we split it in to two parts, with and without [redacted] and [redacted] they've funded the total amount with [redacted]

[redacted], can your lab start new [redacted] work? If so, would be good if we all were to discuss which experiments to do on which [redacted] Some of our joint thinking at the time re the [redacted] work too.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]

Subject: RE: Science rejected the manuscript- need advice re next steps.
Date: Wednesday, June 3, 2020 9:19:01 PM

Sounds good – thank you, [redacted]

From: [redacted]
Sent: Wednesday, June 3, 2020 6:13 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Science rejected the manuscript- need advice re next steps.

Others agreed too….so we will go to STM

[redacted]

From: [redacted]
Sent: Wednesday, June 03, 2020 6:29 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Science rejected the manuscript- need advice re next steps.

I agree with [redacted]
I would send it to STM.

[redacted]

From: [redacted]
Sent: Thursday, June 4, 2020 7:24 AM
To: [redacted]
Cc: [redacted]
Subject: Re: Science rejected the manuscript- need advice re next steps.

Hi [redacted], my personal vote would be take them up on their offer, STM is an excellent journal and it looks like they have opened the door wide open. I'm sure we could add additional translational discussion if they review it as needing any. I'm also not certain how much time we have left on our side for competing reviews. Just my 2 cents. Best, [redacted]

—
[redacted]

On Jun 3, 2020, at 6:13 PM, [redacted] wrote:

Dear colleagues,

Unfortunately, as per below, our manuscript has been triaged out at Science. However, it appears that their sister journal, Science Translational Medicine "would welcome submission of your manuscript for consideration".

Before we go forward, I just wanted to get your suggestions re next steps. We could shoot high and send to Nature first, and then if rejected, back-up to Science Translational Medicine. Alternatively, given the quick read at Science, we could assume same outcome at

Nature, and go directly to Science Translational Medicine.

Let me know your thoughts by tomorrow if possible.

Thanks

[redacted]

……
03-Jun-2020

Dear [redacted]

Manuscript number: abd0733

Thank you for submitting your manuscript "[redacted] to Science. Unfortunately your manuscript was not given a sufficiently high priority rating during the initial screening process, and we are not able to proceed to in-depth review. The overall view is that while your paper will be of great interest to the field it is not one of the most competitive in terms of manuscripts we currently have.

I have personally discussed with the Editors at our sister journal Science Translational Medicine (www.sciencetranslationalmedicine.org) who have indicated that they would welcome submission of your manuscript for consideration. The Editor I have discussed with is Dr. Orla Smith (osmith@aaas.org) who is in charge of the journal. Science Translational Medicine is a high-level, interdisciplinary journal that publishes research that makes significant progress toward improvements in clinical practice. It is not necessary to reformat your paper to have it considered, and we would be happy to transfer your submission from Science to Science Translational Medicine.

If you would like to take advantage of the transfer opportunity, please click here (once you click the link, you'll be directed to a webpage to confirm your decision): https://cts.sciencemag.org/scc/#/action/article/efe8d563-8557-45f1-8b85-2df8eebdad17/transfer/168af0d4-2890-45e2-b841-0fefbb57fe79?includeReviews=false&token=5485e8e1-a5e4-11ea-ac0c-0a26b3007c43.

We now receive many more interesting papers than we can publish. We therefore send for in-depth review only those papers most likely to be ultimately published in Science. Papers are selected on the basis of discipline, novelty, and general significance, in addition to the usual criteria for publication in specialized journals. Therefore, our decision is not a reflection of the quality of your research but rather of our stringent space limitations.

Sincerely,

Priscilla N. Kelly, Ph.D.
Biomedicine Editor
Science

[redacted]

From: [redacted]
To: [redacted]
Subject: RE: Transmission studies
Date: Wednesday, June 24, 2020 5:40:00 AM

OK. Thanks, [redacted]
I will let him know.

[redacted]

From: [redacted]
Sent: Wednesday, June 24, 2020 7:35 PM
To: [redacted]
Subject: RE: Transmission studies

Hi [redacted] Say Yes, Tell him your getting isogenic recombinant viruses from me constructed in the Seattle backbone. [redacted]

From: [redacted]
Sent: Tuesday, June 23, 2020 7:00 PM
To: [redacted]
Cc: [redacted]
Subject: FW: Transmission studies

[redacted],

How should I respond?
He asked the same question during CEIRS/COVID-19 call this morning (US time).

[redacted]

From: [redacted]
Sent: Tuesday, June 23, 2020 11:43 PM
To: [redacted]
Subject: Transmission studies

Dear [redacted]

I apologize if I've already asked you this before, but are you planning any transmission studies that will compare the D614G spike variant with Wuhan-like isolates containing D614? I'm asking because

there are a lot of questions following the recent Scripps study suggesting that the D614G increases infectivity in vitro.

Thanks,

[redacted]

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies
Date: Friday, July 31, 2020 3:10:00 PM

Dear [redacted]

Yes. As soon as we get the tissue titer data, let's have a call.

[redacted]

From: [redacted]
Sent: Saturday, August 1, 2020 4:53 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Hi [redacted] we should set up a call next week so that we can share all of the data regarding this virus. Hope you have a nice weekend. [redacted]

From: [redacted]
Sent: Friday, July 31, 2020 3:48 PM
To: [redacted]
Cc: [redacted] >
Subject: RE: Transmission studies

Thanks, [redacted]
We have also started an experiment to observe just body weight change for over 10 days.

I will share the data with [redacted].

[redacted]

From: [redacted]
Sent: Saturday, August 1, 2020 4:45 AM
To: [redacted]

Cc: [redacted]
Subject: RE: Transmission studies

Hi [redacted], very interesting indeed. I have no problem with you sharing the data. Is the tissue culture seattle strain cause weight loss or not? [redacted]

From: [redacted]
Sent: Friday, July 31, 2020 3:21 PM
To: [redacted]
Cc: [redacted]
Subject: FW: Transmission studies

Dear [redacted]

[redacted] wants an update on the S-614 studies (see below). Here is what I plan to tell him; I hope it is okay with you. I will let you know the virus titers as soon as I get them:

We infected hamsters with 1000 pfu of SARS-CoV-2 strains that differ only at position S-614, generated by [redacted] based on a Seattle isolate, and sacrificed them on days 3 and 6.

The body weight changes of the animals at 3 and 6 days after infection are shown in the attached slide.

Animals infected with the virus bearing S-614G appear to have lost more weight. Titration of lung and nasal turbinate samples from each group on Days 3 and 6 was performed yesterday; we will have the titer results on Monday.

Best,
[redacted]

From: [redacted]
Sent: Saturday, August 1, 2020 1:38 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Hi [redacted]

I hate to bother you (and apologize if you've already gotten this question from others), but I was wondering if you have an estimate of when you think studies of the D614G mutant in hamsters may be complete?

Thanks,
[redacted]

From: [redacted]
Sent: Wednesday, June 24, 2020 7:06 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Dear [redacted],

[redacted] has generated isogenic recombinant SARS-CoV-2 viruses (S-D614 and S-G614) based on the Seattle isolate and is sending them to us. We will be testing them in hamsters once we get them.

Best,
[redacted]

[redacted]

From: [redacted]
Sent: Tuesday, June 23, 2020 11:43 PM
To: [redacted]
Subject: Transmission studies

Dear [redacted]

I apologize if I've already asked you this before, but are you planning any transmission studies that will compare the D614G spike variant with Wuhan-like isolates containing D614? I'm asking because there are a lot of questions following the recent Scripps study suggesting that the D614G increases infectivity in vitro.

Thanks,

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies
Date: Friday, July 31, 2020 6:46:00 PM

Dear [redacted]

I do not know who is on the [redacted] preclinical team. But, we plan to repeat the experiment just to make sure the data are sound.

We are modifying the cages for the transmission experiments. We expect to have results in 3 weeks.

Best,
[redacted]

From: [redacted]
Sent: Saturday, August 1, 2020 6:39 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Dear [redacted]

Thanks so much for sharing your preliminary data. Admittedly, I'm a little surprised! We would be very interested in seeing the titers when you have them.

This may affect decisions we make about challenge stocks for animal experiments. Please let me know if/when you would be comfortable with me sharing the data within the [redacted] preclinical team.

Finally, I can't help asking when you might have data on any transmission differences?

Again, thanks so much. I really appreciate it.

[redacted]

From: [redacted]

Sent: Friday, July 31, 2020 3:50 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Dear [redacted],

We infected hamsters with 1000 pfu of SARS-CoV-2 strains that differ only at position S-614, generated by [redacted] based on a Seattle isolate, and sacrificed them on days 3 and 6.

The body weight changes of the animals for 3 and 6 days after infection are shown in the attached slide.
Animals infected with the virus bearing S-614G appear to have lost more weight.

Titration of lung and nasal turbinate samples from each group on Days 3 and 6 was performed yesterday; we will have the titer results on Monday.

Best,
[redacted]

[redacted]

From: [redacted]
Sent: Saturday, August 1, 2020 1:38 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Hi [redacted]

I hate to bother you (and apologize if you've already gotten this question from others), but I was wondering if you have an estimate of when you think studies of the D614G mutant in hamsters may be complete?

Thanks,
[redacted]

From: [redacted]
Sent: Wednesday, June 24, 2020 7:06 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Transmission studies

Dear [redacted]

[redacted] has generated isogenic recombinant SARS-CoV-2 viruses (S-D614 and S-G614) based on the Seattle isolate and is sending them to us. We will be testing them in hamsters once we get them.

Best,
[redacted]

[redacted]

From: [redacted]
Sent: Tuesday, June 23, 2020 11:43 PM
To: [redacted]
Subject: Transmission studies

Dear [redacted]

I apologize if I've already asked you this before, but are you planning any transmission studies that will compare the D614G spike variant with Wuhan-like isolates containing D614? I'm asking because there are a lot of questions following the recent Scripps study suggesting that the D614G increases infectivity in vitro.

Thanks,

[redacted]

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call agenda for tomorrow (Monday)
Date: Monday, July 6, 2020 9:21:17 AM
Attachments: image001.png
image002.png
image003.png

Apologies for our early departure from the call today. Somehow, our agenda had a 1-hour time slot for the [redacted] call and the secretariat added a second call right next to it.

Yours sincerely,

[redacted]

[redacted]

[redacted]

CC: [redacted]

[redacted] call agenda for tomorrow (Monday)

[redacted]

Re the agenda for our meeting tomorrow. After general updates that will likely not take much time, I suggest we get into some planning discussions. We have been working on reanalyzing existing data, we could give an update on that and from there go into a discussion on planning. A rough outline of possibilities below, it will make more sense with discussion (we hope).

[redacted]

[redacted] for a subset of these viruses:

- [redacted] maybe the cell version of the current vaccine
- [redacted]
- [redacted]

We had also been thinking of some other experiments to add to the discussion of what our priorities should be:

- [redacted]

- [redacted]. For example, [redacted] (early, middle, and lates in other lineages), and test against human sera that we select from the human-sera experiment on that cluster.

- [redacted] work, looking at the [redacted]

- The pilot we have all discussed of [redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: M[redacted] call debrief
Date: Thursday, June 4, 2020 2:28:42 PM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png
image007.png
image008.png
image009.png
image010.png
image011.png
image012.png

You can put what I wrote in an email, but [redacted], and I would not put anything to definitive in [redacted] mouth as he was not informed he would be quoted.

[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
[redacted]
[redacted]
CC: [redacted]
[redacted]
[redacted]: Re: [redacted] call debrief

i hear that [redacted], and it makes sense. especially as we don't know the [redacted]

do others concur? and if so, what are your thoughts re me, or all of us talking with [redacted]? or an email to them. i think me sending an email to them, with us copied, would be the better than a call, they just need the info, then they can have a think, and maybe a call after they've discussed internally. what do others think?

[redacted] how much of what you discussed with [redacted] do you think it is ok to put in an email?

[redacted]

On Thu, Jun 4, 2020 at 4:22 PM [redacted] wrote:

> I would have a preference to work with [redacted] so would prefer to talk with [redacted] first about extra funds. If they suggest we should go strengthen the [redacted] team and help them spend their money wisely, than I would reconsider

Yours sincerely,

CC: **:** Re call debrief

Thanks for all this . Very helpful.

I add a little extra info re as you know is doing a you had been helpful when was looking around re who might be possible at After trying a couple of labs to see how flexible they would be with the arrangement with us, she settled on one that non of us had identified previously, the lab of . He is one of the consortium, perhaps I mentioned that when we had our call.

recently extended previous ideas she'd had and come up with an That would require making constructs that . She presented that at our lab meeting on Tuesday, and said that that is right in the research area of the lab, that the lab is currently pathogen-agnostic, but working on basic structural methods, and is very open to collaboration, and through knows of our work (not anything that is not already public knowledge).

In a follow up call with she also thinks that the

What do people think as to whether it is worth a call with the people, or first, before we go back to ?

On Thu, Jun 4, 2020 at 3:52 PM wrote:

> I spoke briefly with about
>
> In general, he said that are a bit more milestone driven than the . Options are both internal and external (so competitive if we come in). and rank the options together. The three are similar in size (funding) and have similar options.
>
> would very much like to work with us, but also thinks our current collective budget is higher than they can handle with

options, as we already knew. If [redacted] bring in EXTRA money, than that could work for him. [redacted] is co-PI so [redacted] would need to discuss with him also, but doesn't see a problem himself.

[redacted] also said that budgets for GMP production and clinical trials would NOT come from the [redacted], and would thus NOT be competitive with their own work. NIH would judge which candidates need to go into production and into clinical trials and pay for it. The sample processing from clinical studies could still go as (relatively smaller) options, e.g. via [redacted]. So, if we could do most of our work via clinical study, and smaller parts via [redacted]) that might work. Still, preferable to bring in extra money as anything we ask for would compete with [redacted] and [redacted].

[redacted] thinks that [redacted]. They have [redacted]. In theory, they could be interested in our concept as they have [redacted]. But [redacted] would very much like to bring us into [redacted], if we do not take too much of their money.

So with that, I think [redacted] can have another call with [redacted] and separately with [redacted]?
We have a place to "land", but the pockets of [redacted] are not deep enough. Any possibility of routing extra money into [redacted]?
BTW [redacted] said that some organization (NCI?) has routed COVID funds into the CIVICs, so it can be done....

Yours sincerely,

[redacted]

[redacted]:
[redacted]
[redacted]
[redacted]
CC: [redacted]
[redacted]: Re: [redacted] call debrief

Then let's not do today.

Guessing the same time constrains given our timezones, please reply with whether you could do the same times Mon Tue or Wed next week.

6 or 7am CT
1 or 2 pm NL
8 or 9pm Tokyo

[redacted]

On Fri, May 22, 2020 at 9:14 AM [redacted] wrote:

I will indeed not be able to join. I will be out of the office the coming week.
Best
[redacted]

From: [redacted]
Sent: Friday, May 22, 2020 9:44:20 AM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call debrief

I will try to connect. [redacted] will – most likely - not.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
CC: [redacted]
[redacted] Re: [redacted] call debrief

[redacted]

Could you do a 30 minute (I estimate) call at 13h or 14h your time today? Or suggest another day/time that works for you please.

The list of CIVICs here https://www.niaid.nih.gov/research/civics

[redacted]

On Thu, May 21, 2020 at 10:58 PM [redacted] > wrote:

> I am available after 6-8 am CT on May 22.

[redacted]

From: [redacted]
Sent: Thursday, May 21, 2020 11:14 PM
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] call debrief

Any time after 5 am CT will be okay.
There is a CEIRS Webinar at 9:30 am CT, but I can skip it.

[redacted]

From: [redacted]
Sent: Thursday, May 21, 2020 9:10 AM
To: [redacted]
Cc: [redacted]
Subject: [redacted] call debrief

I suggest we have a zoom debrief from this call tomorrow.

To discuss which center to approach, and how to approach.

Please email when you could do a 30 minute Zoom tomorrow so we can set a time. I can make any time work.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: hCK cell line
Date: Monday, June 29, 2020 3:57:46 AM
Attachments: image001.png
image002.png
image003.png

Thanks [redacted],

We will get in touch with [redacted] or [redacted].

All the best,

[redacted]

[redacted]

From: [redacted]
Date: Monday, 29 June 2020 at 09:40
To: "[redacted]
Cc: [redacted]
Subject: Re: hCK cell line

Hi [redacted],
Yes, that is fine by me. [redacted] or [redacted] can provide them.
Kind regards
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
CC: [redacted]
[redacted]**:** hCK cell line

Hi [redacted]

We're working with some recent H3 viruses and had contacted [redacted] about using his hCK cell line. We have completed the relevant MTA and now are now ready to start using it.

To save the costs of shipping from the US, would it be possible to get the line from you? [redacted] has indicated that this would be fine with him if it was agreeable to you. If yes, [redacted] from my group or [redacted] could collect them from you in Rotterdam to save you shipping costs too.

All the best,

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Call with [redacted] and [redacted]
Date: Sunday, May 10, 2020 5:43:00 PM
Attachments: image001.png
image002.png
image003.png

Thank you for this detailed update, [redacted]
I am glad that you were able to talk to [redacted]. I think this call was very important to keep [redacted] thinking about our flu work during the COVID-19 crisis. I like the plan, which will ensure that we continue to have dialogues with [redacted] regarding funding.
[redacted]

From: [redacted]
Sent: Monday, May 11, 2020 3:52 AM
To: [redacted]
Cc: [redacted]
Subject: Re: Call with [redacted] and [redacted]

Sounds great [redacted]. As positive as could be, with covid-19 unfortunately an obstacle.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
CC: [redacted]
[redacted]**:** Call with [redacted] and [redacted]

Just got off a call with [redacted] and [redacted] I'll simply transcribe my notes.

- This is one of the very few calls on anything other than COVID-19 that we've had in month

- They wondered what NIAID have said to us [redacted] and [redacted] have not replied yet)

- They can't think of a better approach than ours for either [redacted]

- Just that the timing is difficult

- They are not allowed to review anything non COVID-19, but they can try to get the discussion started if we put in a 3, 4, or 5 page concept that they will then circulate with [redacted]. Put everything in it, [redacted]

- [redacted] jury is out, especially after vaccitech's results https://www.vaccitech.co.uk/phase-2-clinical-results-for-vaccitech-universal-influenza/

- Putting the [redacted] proposal together is a sure-fire way to see if NIAID want to fund [redacted] just like last time.

- [redacted] at the moment, they don't know what will happen, maybe even [redacted] will come back.

- Our concept should be for what should be being done for flu vaccines. 1st para should be high level, and needs to grab attention, especially in the current times when it is very difficult to get any attention on flu.

- Will be interesting for them to see what it will look like to see a relatively modest concept like ours that can have so much impact as the other concepts they have are at $400m

- [redacted] and [redacted] are running a > $1b portfolio on COVID-19

- [redacted]

- They will share our concept with [redacted].

- I reckon we should coordinate with [redacted] re our concept before submitting to [redacted], maybe we submit it to all three? (This not discussed with [redacted] I just realised this now).

- We should submit the paperwork for a no-cost extension to the current [redacted] contract of 6 months. Not for CVV manufacturing, that should go in the new concept. But to give us more time to complete because of lab shutdowns. Will be at least a month before the contracts people at [redacted] can look at it.

- Will be 6 months to a year before [redacted] can fund anything but COVID-19

[redacted] seemed relaxed, interested, and like he definitely wanted the work on [redacted] to continue. It is just a timing issue.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Draft agenda for [redacted] virtual meeting
Date: Wednesday, April 22, 2020 5:16:19 AM
Attachments: image001.png
image002.png
image003.png

Dear All,

I am fine either way.
While everybody has COVID-19 on their mind right now, it may also be important to keep flu on the radar, for the reasons already spelled out by the others.

Best,

[redacted]

From: [redacted]
Sent: Wednesday, April 22, 2020 4:57 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Draft agenda for [redacted] virtual meeting

I am also fine either way. But, first, I think they are preoccupied with COVID-19. Second, [redacted] is not going to consider flu funding for a while. Third, by the time they are ready to consider future funding of our project, they will not remember what we discussed on Friday. For these reasons, I am more inclined to postpone. At the same time, we will run out of the money needed to keep our people. This may be the most critical issue to discuss with [redacted], rather than the science. That is, unless we get some funds to keep our people, by the time [redacted] is ready to consider the next phase of our project, we may have to let some people go and we may not be as efficient as we were when our project resumes.
Just a thought. But, I am fine with any approaches you decide. I will try hard to keep my eyes open until 2am!

Best,
[redacted]

From: [redacted]
Sent: Wednesday, April 22, 2020 4:02 PM
To: [redacted]
Cc: [redacted]
Subject: Re: Draft agenda for [redacted] virtual meeting

Either way is fine by me. We have just been told our live does not return to normal for the next month.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
CC: [redacted]
[redacted]: Re: Draft agenda for [redacted] virtual meeting

Our other option is to suggest we delay by one month. Please let us know your thoughts.

[redacted]

On Tue, Apr 21, 2020 at 10:44 PM [redacted] wrote:

> Dear all,
>
> Please find below our proposed agenda. Please let us know your thoughts?
>
> 08:30-08:40 Introductions
> 08:40-09:40 [redacted] for everyone)
> 09:40-10:00 [redacted]
> 10:00-10:05 Break
> 10:05-11:05 [redacted] for everyone)
> 11:25-11:45 [redacted]
> 11:45-12:00 General discussion
>
> Some additional information to think about based on a call I had with [redacted] today:
>
> [redacted] also requested a time reduction so we have reduced to 3.5 hours from 4.5 [redacted] request stemming from a significant number of covid related meetings and some people having to likely come off the call early. Do you think 3.5 hours is short enough?
>
> [redacted] also thinks it's unlikely [redacted] will be present because he's on CDC's rotation for covid at present .
>
> Also that [redacted] will be out from 10-11 am (re any immunology discussions).

Another key point is that [redacted] are not allowed to review any non covid ie. Flu, proposals currently. This is effective until the covid pandemic "goes away". I believe [redacted] tends to discuss this issue during the last part of the call.

Many thanks

[redacted]

From: [redacted]
To: [redacted]
Subject: RE: I hope I did not make any mistakes and did just to all of your work with my presentation
Date: Friday, April 24, 2020 8:54:00 AM

It was very clear!

Thank you, [redacted].

[redacted]

From: [redacted]
Sent: Friday, April 24, 2020 10:53 PM
To: [redacted]
Subject: I hope I did not make any mistakes and did just to all of your work with my presentation

> I hope I did not make any mistakes and did just to all of your work with my presentation
> [redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Introducing [redacted] re. Animal infections/OIE
Date: Saturday, May 9, 2020 7:54:00 AM

Just a quick update: our letter to the editor will be published online at NEJM.org at 5:00 PM ET on Wednesday, May 13, 2020.

Best,

[redacted]

From: [redacted]
Sent: Monday, May 4, 2020 8:58 PM
To: [redacted]
Cc: [redacted]
Subject: Re: Introducing [redacted] re. Animal infections/OIE

Hi [redacted], so nice to hear from you again, its been a while and I was glad to hear that you're working with [redacted] now.

It would be great to share any early results that you may be able to disclose with the [redacted] working group that I lead, and both WHO [redacted] [redacted] and [redacted]) and FAO participate in the weekly/biweekly meetings.

Ultimately, public concerns will depend on how the popular press or media describe the results. We have already seen abandonment of pet cats and dogs from fear of catching COVID-19. We know that some animals can produce significant viral loads and transmit among their own species (cats, mink, ferrets) but to date no one has documented human infection from an animal. I would strongly suggest that that statement be included in any findings to put the situation in perspective and authors take into consideration actions that are practical from their findings and also try to avoid unintended consequences. Scientists tend to talk about the "possible" but the public mis-interprets that to mean "dangerous."

Hope you are well and look forward to hearing more of your recent work.

All the Best,

[redacted]

[redacted]

[redacted]

[redacted]

On May 3, 2020, at 6:37 PM, wrote:

Thanks,
I contacted at the WHO and she introduced me who is leading the group working on COVID-19 and human-animal interface at the WHO.

I would appreciate any suggestions you may have to avoid public panic about our findings.

From:
Sent: Monday, May 4, 2020 6:19 AM
To:
Cc:
Subject: Introducing re. Animal infections/OIE
Importance: High

Hi

On the NIAID call last week I mentioned that I'd introduce you to is our Exec. VP for Science & Policy, is group, and a member of their taskforce on COVID-19. was previously part of the CEIRS network when he was at and you may remember his work managing the

[redacted]. He also was the [redacted]

[redacted] been very active within OIE/WHO/FAO managing their outreach on animal infections with COVID, including working with [redacted] on how to get good information out about the first dog infection, and recently on issues around domestic cats, tigers etc. in zoos, mink in farms.

[redacted] described some really important work on cats that he's getting close to publishing. It's important because, unlike previous papers, it shows that cats can transmit among cats, but that 1) their viral titers suggest they might also be able to infect people; and 2) some of these cats are essentially asymptomatic.

I hope you'll both be able to talk briefly so [redacted] can get ready with the OIE for statements if there is a strong public reaction when the paper comes out (people love cats!).

Cheers,

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Subject: RE: Is there anything I forgot to mention that I could add when we return?
Date: Friday, April 24, 2020 10:01:00 AM

I think you covered everything.

From: [redacted]
Sent: Friday, April 24, 2020 11:51 PM
To: [redacted]
Subject: Is there anything I forgot to mention that I could add when we return?

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: RE:
Date: Monday, May 11, 2020 7:00:00 AM

I will call him. Can I have his phone number?

[redacted]

From: [redacted]
Sent: Monday, May 11, 2020 9:00 PM
To: [redacted]
Cc: [redacted]
Subject: RE: RE:

That works. Do you want me to send a zoom or can you call his cell phone?

From: [redacted]
Sent: Monday, May 11, 2020 7:59 AM
To: [redacted]
Cc: [redacted]
Subject: RE: RE:

Thanks, [redacted]
How about 5:30pm ET on May 14?
[redacted]

From: [redacted]
Sent: Monday, May 11, 2020 8:56 PM
To: [redacted]
Subject: RE:

Hi [redacted]
[redacted] is available:
Wednesday 5/13 after 4:30 ET
5/14 2:30-4 and after 5

Do these ranges work for you?
[redacted]

From: [redacted]
Sent: Monday, May 11, 2020 7:53 AM

To: [redacted]
Cc: [redacted]
Subject: RE:

Hi [redacted], I 'll be glad to chat. Lets see if [redacted] can find a time. Hope your doing well. [redacted]

From: [redacted]
Sent: Monday, May 11, 2020 5:49 AM
To: [redacted]
Cc: [redacted]
Subject:

Dear [redacted],

Are you collaborating with anyone to analyze mutant SARS-CoV-2 strains?
We have a hamster model running and could test any mutants you may have or plan to create. I have some ideas, but I am sure you already thought about them.

Best,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: RE:
Date: Monday, May 11, 2020 6:54:00 AM

Great!

I am currently in Japan (since March 20 due to travel restrictions).
So, it is easier if we can talk your late afternoon.

Thanks,
[redacted]

From: [redacted]
Sent: Monday, May 11, 2020 8:53 PM
To: [redacted]
Cc: [redacted]
Subject: RE:

Hi [redacted] I 'll be glad to chat. Lets see if [redacted] can find a time. Hope your doing well. [redacted]

From: [redacted]
Sent: Monday, May 11, 2020 5:49 AM
To: [redacted]
Cc: [redacted]
Subject:

Dear [redacted],

Are you collaborating with anyone to analyze mutant SARS-CoV-2 strains?
We have a hamster model running and could test any mutants you may have or plan to create. I have some ideas, but I am sure you already thought about them.

Best,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Talking with [redacted] on Sunday
Date: Saturday, May 9, 2020 6:10:49 AM

I don't have any additional thoughts either.

Thanks,

[redacted]

From: [redacted]
Sent: Saturday, May 9, 2020 4:14 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Talking with [redacted] on Sunday

I do not have any additional thoughts.

[redacted]

From: [redacted]
Sent: Saturday, May 9, 2020 5:13 PM
To: [redacted]
Cc: [redacted]
Subject: Talking with [redacted] on Sunday

I'm talking with [redacted] and [redacted] on Sunday at 4pm [redacted] time.

If prior to that you have any additional thoughts post our strategy discussion last Monday please let me know.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted]
Date: Friday, May 22, 2020 7:57:00 AM

I see. I hope we find a way to continue our work.

[redacted]

From: [redacted]
Sent: Friday, May 22, 2020 9:45 PM
To: [redacted]
Cc: [redacted]
Subject: Fwd: [redacted]

We don't get to write a full proposal to [redacted]. I'm surprised.

Thanks all for your help pulling the preliminary proposal together.

[redacted]

---------- Forwarded message ---------
From: [redacted]
Date: Fri, May 22, 2020 at 12:28 PM
Subject: [redacted]
To: [redacted]

Dear [redacted]

Reference number: [redacted]

Thank you for your recent preliminary application for a [redacted].

We have now considered the preliminary applications for the current competition and I am sorry to tell you that your proposal was not shortlisted for further consideration. The applications were assessed for a number of criteria, including the strength of the research question; the articulated need for a collaborative approach and the track records of the applicants.

There was a great deal of interest in the scheme and a large number of high quality applications were received. I regret that, when viewed in competition with the other applications, your submission was not chosen to go forward for further consideration.

I realise that this decision will come as a disappointment and hope that you will be able to obtain support from elsewhere. I would be grateful if you could convey this decision to the other applicants.

If you have any questions, please do not hesitate to contact me

Yours sincerely

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: hACE2 mice
Date: Monday, May 25, 2020 3:40:00 PM

I take anything you can provide.

Thanks!

[redacted]

From: [redacted]
Sent: Tuesday, May 26, 2020 2:46 AM
To: [redacted]
Cc: [redacted]
Subject: Re: hACE2 mice

[redacted] asks what about homozygous breeders?

Sent from Outlook Mobile

From: [redacted]
Sent: Monday, May 25, 2020 1:44:10 PM
To: [redacted]
Cc: [redacted]
Subject: RE: hACE2 mice

Hi [redacted],
We still have an unresolved problem with not all hACE2 hets infecting (roughly 50% have virus at D2). I recommend against sending any more mice out before we figure this issue out.
[redacted]

From: [redacted]
Sent: Monday, May 25, 2020 10:48 AM
To: [redacted]
Cc: [redacted]
Subject: RE: hACE2 mice

Hi [redacted] and [redacted] Can we get some brreders to [redacted] asap? Thanks [redacted]

From: [redacted]
Sent: Sunday, May 24, 2020 4:43 PM
To: [redacted]

Cc: [redacted]>
Subject: hACE2 mice

Dear [redacted]

Do you know when we can get your hACE2 mice?

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call on Monday
Date: Friday, May 29, 2020 10:02:11 AM

Monday is a bank holiday for me. Happy to postpone.
[redacted]

From: [redacted]
Sent: Friday, May 29, 2020 5:00 PM
To: [redacted]
Cc: [redacted]
Subject: [redacted] call on Monday

Our monthly partners call is scheduled for this Monday at the usual time.

We have some work to present, but it is not urgent. We are still in the middle of it, so on one hand it would go faster if we presented it a bit later when we have more results, on the other hand it would be great to get your thoughts now.

[redacted], if you are still very busy with corona work, perhaps you'd prefer to not have the call?

[redacted] perhaps you will still be away?

[redacted], you have already heard what we will present.

Please let me know whether you prefer to have our call on usual Monday.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: Call with [redacted] and [redacted]
Date: Sunday, May 10, 2020 1:51:45 PM
Attachments: image001.png
image002.png
image003.png

Sounds great [redacted]. As positive as could be, with covid-19 unfortunately an obstacle.
[redacted]

Yours sincerely,

[redacted]

[redacted]
CC: [redacted]
[redacted] Call with [redacted] and [redacted]

Just got off a call with [redacted] and [redacted]. I'll simply transcribe my notes.

- This is one of the very few calls on anything other than COVID-19 that we've had in month

- They wondered what NIAID have said to us [redacted] and [redacted] have not replied yet)

- They can't think of a better approach than ours for either [redacted]

- Just that the timing is difficult

- They are not allowed to review anything non COVID-19, but they can try to get the discussion started if we put in a 3, 4, or 5 page concept that they will then circulate with [redacted]. Put everything in it, [redacted]

- [redacted] jury is out, especially after vaccitech's results https://www.vaccitech.co.uk/phase-2-clinical-results-for-vaccitech-universal-influenza/

- Putting the [redacted] proposal together is a sure-fire way to see if NIAID want to fund [redacted], just like last

time.

- [redacted] at the moment, they don't know what will happen, maybe even [redacted] will come back.

- Our concept should be for what should be being done for flu vaccines. 1st para should be high level, and needs to grab attention, especially in the current times when it is very difficult to get any attention on flu.

- Will be interesting for them to see what it will look like to see a relatively modest concept like ours that can have so much impact as the other concepts they have are at $400m

- [redacted] and [redacted] are running a > $1b portfolio on COVID-19

- [redacted].

- They will share our concept with [redacted].

- I reckon we should coordinate with NIAID and CDC re our concept before submitting to [redacted], maybe we submit it to all three? (This not discussed with [redacted], I just realised this now).

- We should submit the paperwork for a no-cost extension to the current [redacted] contract of 6 months. Not for CVV manufacturing, that should go in the new concept. But to give us more time to complete because of lab shutdowns. Will be at least a month before the contracts people at [redacted] can look at it.

- Will be 6 months to a year before [redacted] can fund anything but COVID-19

[redacted] seemed relaxed, interested, and like he definitely wanted the work on [redacted] to continue. It is just a timing issue.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: Catch up after our annual update meeting
Date: Wednesday, May 13, 2020 1:05:15 AM
Attachments: image001.png
image002.png
image003.png

Both days OK for me.

[redacted], please see below, how about you?

[redacted]

On Wed, May 13, 2020 at 6:33 AM [redacted] wrote:

> I can make it on both days, with a preference for the 21st.
>
> [redacted]
>
> Yours sincerely,
>
> [redacted]
>
> [redacted]
>
> ---
>
> [redacted]>
> [redacted]
> [redacted] FW: Catch up after our annual update meeting
>
> I am available on May 21 9am ET and May 22 9am ET.

[redacted]

From: [redacted]
Sent: Wednesday, May 13, 2020 9:28 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Catch up after our annual update meeting

Hi [redacted],

Thanks for sending the presentations to us. We're sorry we couldn't stay the whole time! It would be good to talk to you all about next steps. Would Thursday May 21st at 9AM ET or Friday May 22nd and 9AM ET work for everyone to speak about the project?

[redacted]

From: [redacted]
Sent: Friday, May 8, 2020 2:38 AM
To: [redacted]
Cc: [redacted]
Subject: Catch up after our annual update meeting

[redacted]

Attached the minutes, and seasonal presentation from our [redacted] annual update meeting a couple of weeks ago. The pandemic presentation is too big for email, you can download it here

[redacted]

Many thanks for the time you could spend with us, we understand you had a meeting you needed to attend part way though our seasonal presentation.

The [redacted] projects are succeeding beyond what even we could have imagined 5 years ago. The [redacted] part you saw, and on the [redacted] endpoint.

There are likely substantial further improvements to the seasonal vaccine that are in the pipeline, but all funding for this work runs out in March next year.

We realize the timing is bad, but wonder whether we could have a call with you on this please.

Best wishes

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: Draft agenda for [redacted] virtual meeting
Date: Wednesday, April 22, 2020 3:04:44 AM
Attachments: image001.png
image002.png
image003.png

Maybe the 1hr presentations should be reduced (e.g. 45 min max), to allow more time for questions and discussion?

Yours sincerely,

[redacted]

[redacted]

[redacted]
CC: [redacted]
[redacted]**:** Draft agenda for [redacted] virtual meeting

Dear all,

Please find below our proposed agenda. Please let us know your thoughts?

08:30-08:40 Introductions
08:40-09:40 [redacted] ([redacted] for everyone)
09:40-10:00 [redacted]
10:00-10:05 Break
10:05-11:05 [redacted] ([redacted] for everyone)
11:25-11:45 [redacted]
11:45-12:00 General discussion

Some additional information to think about based on a call I had with [redacted] today:

[redacted] also requested a time reduction so we have reduced to 3.5 hours from 4.5. [redacted] request stemming from a significant number of covid related meetings and some people having to likely come off the call early. Do you think 3.5 hours is short enough?

[redacted] also thinks it's unlikely [redacted] will be present because he's on CDC's rotation for covid at present .

Also that [redacted] will be out from 10-11 am (re any immunology discussions).

Another key point is that [redacted] are not allowed to review any non covid ie. Flu, proposals currently. This is effective until the covid pandemic "goes away". I believe [redacted] tends to discuss this issue during the last part of the call.

Many thanks

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: Draft agenda for [redacted] virtual meeting
Date: Wednesday, April 22, 2020 2:40:56 AM
Attachments: image001.png
image002.png
image003.png

Not easy, ideally we'd like them to have some time to discuss our future plans, but in a month, it is very unlikely that the storm will have passed. It is maybe better to have the meeting now, and if necessary another one when things have gone a bit more to normal?
Cheers
[redacted]

From: [redacted]
Date: Wednesday, 22 April 2020 at 09:02
To: '[redacted]
Cc: [redacted]
Subject: Re: Draft agenda for [redacted] virtual meeting

Either way is fine by me. We have just been told our live does not return to normal for the next month.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted] >
[redacted]
[redacted]
CC: [redacted]
[redacted]**:** Re: Draft agenda for [redacted] virtual meeting

Our other option is to suggest we delay by one month. Please let us know your thoughts.

[redacted]

On Tue, Apr 21, 2020 at 10:44 PM [redacted] wrote:

> Dear all,
>
> Please find below our proposed agenda. Please let us know your thoughts?
>
> 08:30-08:40 Introductions
> 08:40-09:40 [redacted] for everyone)
> 09:40-10:00 [redacted]
> 10:00-10:05 Break
> 10:05-11:05 [redacted] for everyone)
> 11:25-11:45 [redacted]
> 11:45-12:00 General discussion
>
> Some additional information to think about based on a call I had with [redacted] today:
>
> [redacted] also requested a time reduction so we have reduced to 3.5 hours from 4.5. [redacted] request stemming from a significant number of covid related meetings and some people having to likely come off the call early. Do you think 3.5 hours is short enough?
>
> [redacted] also thinks it's unlikely [redacted] will be present because he's on CDC's rotation for covid at present .
>
> Also that [redacted] will be out from 10-11 am (re any immunology discussions).
>
> Another key point is that [redacted] are not allowed to review any non covid ie. Flu, proposals currently. This is effective until the covid pandemic "goes away". I believe [redacted] tends to discuss this issue during the last part of the call.
>
> Many thanks
>
> [redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call debrief
Date: Friday, May 22, 2020 3:56:02 AM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

I could do all 3 days at these times. [redacted] will probably not join.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
[redacted]
[redacted]
CC: [redacted]
[redacted]
[redacted]**:** Re: [redacted] call debrief

Then let's not do today.

Guessing the same time constrains given our timezones, please reply with whether you could do the same times Mon Tue or Wed next week.

6 or 7am CT
1 or 2 pm NL
8 or 9pm Tokyo

[redacted]

On Fri, May 22, 2020 at 9:14 AM [redacted] > wrote:

> I will indeed not be able to join. I will be out of the office the coming week.
> Best

[redacted]

From: [redacted] >
Sent: Friday, May 22, 2020 9:44:20 AM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call debrief

I will try to connect. [redacted] will – most likely - not.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
[redacted] >
CC: [redacted]
[redacted]**:** Re: [redacted] call debrief

[redacted]

Could you do a 30 minute (I estimate) call at 13h or 14h your time today? Or suggest another day/time that works for you please.

The list of CIVICs here https://www.niaid.nih.gov/research/civics

[redacted]

On Thu, May 21, 2020 at 10:58 PM [redacted] > wrote:

> I am available after 6-8 am CT on May 22.
>
> [redacted]

I

From: [redacted]
Sent: Thursday, May 21, 2020 11:14 PM
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] call debrief

Any time after 5 am CT will be okay.
There is a CEIRS Webinar at 9:30 am CT, but I can skip it.

[redacted]

From: [redacted]
Sent: Thursday, May 21, 2020 9:10 AM
To: [redacted]
Cc: [redacted]
Subject: [redacted] call debrief

I suggest we have a zoom debrief from this call tomorrow.

To discuss which center to approach, and how to approach.

Please email when you could do a 30 minute Zoom tomorrow so we can set a time. I can make any time work.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call debrief
Date: Sunday, May 24, 2020 6:53:16 AM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

Thanks [redacted].

We'll catch [redacted] up when she is back.

[redacted]

On Sun, May 24, 2020 at 12:42 PM [redacted] wrote:

> OK, [redacted]
>
> [redacted]
>
> ---
>
> **From:** [redacted]
> **Sent:** Sunday, May 24, 2020 8:31 PM
> **To:** [redacted]
> **Cc:** [redacted]
> [redacted]>
> **Subject:** Re: [redacted] call debrief
>
> Thanks [redacted]
>
> Let's do Wednesday please, 6am CT, 1pm NL, 8pm Tokyo.
>
> [redacted] we've not heard from you, can that work for you too?
>
> [redacted]
>
> On Fri, May 22, 2020 at 12:04 PM [redacted]> wrote:
>
> > I can do all 3 days too.
> >
> > ---
> >
> > **From:** [redacted]
> > **Sent:** Friday, May 22, 2020 5:56 PM
> > **To:** [redacted]>
> > **Cc:** [redacted]
> > **Subject:** Re: [redacted] call debrief

I could do all 3 days at these times. [redacted] will probably not join.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]>
[redacted]
CC: [redacted]
[redacted] Re: [redacted] call debrief

Then let's not do today.

Guessing the same time constrains given our timezones, please reply with whether you could do the same times Mon Tue or Wed next week.

6 or 7am CT

1 or 2 pm NL

8 or 9pm Tokyo

[redacted]

On Fri, May 22, 2020 at 9:14 AM [redacted] > wrote:

I will indeed not be able to join. I will be out of the office the coming week.

Best

[redacted]

From: [redacted]
Sent: Friday, May 22, 2020 9:44:20 AM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call debrief

I will try to connect. [redacted] will – most likely - not.

[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted] >
CC: [redacted] Re: [redacted] call debrief

[redacted]

Could you do a 30 minute (I estimate) call at 13h or 14h your time today? Or suggest another day/time that works for you please.

The list of CIVICs here https://www.niaid.nih.gov/research/civics

[redacted]

On Thu, May 21, 2020 at 10:58 PM [redacted] > wrote:

I am available after 6-8 am CT on May 22.

[redacted]

|

From: [redacted]
Sent: Thursday, May 21, 2020 11:14 PM
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] call debrief

Any time after 5 am CT will be okay.

There is a CEIRS Webinar at 9:30 am CT, but I can skip it.

[redacted]

From: [redacted]
Sent: Thursday, May 21, 2020 9:10 AM
To: [redacted]
Cc: [redacted]
Subject: [redacted] call debrief

I suggest we have a zoom debrief from this call tomorrow.

To discuss which center to approach, and how to approach.

Please email when you could do a 30 minute Zoom tomorrow so we can set a time. I can make any time work.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] call debrief
Date: Friday, May 22, 2020 3:11:29 AM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

That would be better for me, if it is not a rush. It is a bank-weekend holiday here. [redacted] is away to [redacted] to deal with family matters and is unlikely to respond.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
CC: [redacted]
[redacted]**:** Re: [redacted] call debrief

[redacted]

Shall we make it a different day? There is not a rush.

[redacted]

On Fri, May 22, 2020 at 8:44 AM [redacted] wrote:

> I will try to connect. [redacted] will – most likely - not.
> [redacted]
>
> Yours sincerely,
>
> [redacted]

[redacted]

[redacted]
CC: [redacted]

[redacted],

Could you do a 30 minute (I estimate) call at 13h or 14h your time today? Or suggest another day/time that works for you please.

The list of CIVICs here https://www.niaid.nih.gov/research/civics

[redacted]

On Thu, May 21, 2020 at 10:58 PM [redacted] wrote:

> I am available after 6-8 am CT on May 22.
>
> [redacted]
> |
>
> ---
>
> **From:** [redacted]
> **Sent:** Thursday, May 21, 2020 11:14 PM
> **To:** [redacted] >
> **Cc:** [redacted]
> **Subject:** RE: [redacted] call debrief
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> There is a CEIRS Webinar at 9:30 am CT, but I can skip it.
>
> [redacted]
>
> **From:** [redacted]
> **Sent:** Thursday, May 21, 2020 9:10 AM
> **To:** [redacted]

Cc: [redacted]
Subject: [redacted] call debrief

I suggest we have a zoom debrief from this call tomorrow.

To discuss which center to approach, and how to approach.

Please email when you could do a 30 minute Zoom tomorrow so we can set a time. I can make any time work.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: New paper from CRIP
Date: Tuesday, April 14, 2020 1:28:19 AM
Attachments: image001.png
image002.png
image003.png
JVI00537-Verhagen et al 2020.pdf

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Phylogeography and antigenic diversity of low pathogenic avian influenza H13 and H16 viruses

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Running title: Genetic and antigenic variation avian influenza virus

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Abstract

Low pathogenic avian influenza viruses (LPAIVs) are genetically highly variable and have diversified into multiple evolutionary lineages that are primarily associated with wild bird reservoirs. Antigenic variation has been described for mammalian influenza viruses and for highly pathogenic avian influenza viruses that circulate in poultry, but much less is known about antigenic variation of LPAIVs. In this study, we focussed on H13 and H16 LPAIVs that circulate globally in gulls. We investigated the evolutionary history and intercontinental gene flow based on the hemagglutinin (HA) gene and used representative viruses from genetically distinct lineages to determine their antigenic properties by hemagglutination inhibition assays. For H13 at least three distinct genetic clades were evident, while for H16 at least two distinct genetic clades were evident. Twenty and ten events of intercontinental gene flow were identified for H13 and for H16 viruses, respectively. At least two antigenic variants of H13 and at least one antigenic variant of H16 were identified. Amino acid positions in the HA protein that may be involved in the antigenic variation were inferred, and some of the positions were located near the receptor binding site of the HA protein, as they are in the HA protein of mammalian influenza A viruses. These findings suggest independent circulation of H13 and H16 subtypes in gull populations as antigenic patterns do not overlap and contribute to the understanding of the genetic and antigenic variation of LPAIV naturally circulating in wild birds.

Importance

Wild birds play a major role in the epidemiology of low pathogenic avian influenza viruses (LPAIVs) from which these viruses are occasionally transmitted—directly or indirectly—to other species, including domestic animals, wild mammals and humans, where they can cause

subclinical to fatal disease. Despite a multitude of genetic studies, the antigenic variation of LPAIVs in wild birds is poorly understood. Here, we investigated the evolutionary history, intercontinental gene flow, and the antigenic variation among H13 and H16 LPAIVs. The circulation of the subtypes H13 and H16 seems to be maintained by a narrower host range, in particular gulls, than for the majority of LPAIV subtypes and may therefore serve as a model for evolution and epidemiology of H1-H12 LPAIVs in wild birds. The findings suggest that H13 and H16 LPAIVs circulate independently of each other and emphasize the need to investigate within clade antigenic variation of LPAIVs in wild birds.

Keywords: avian viruses, influenza, evolution, epidemiology, ecology, antigenic variation, seabird

62 **Introduction**

63

64 Wild birds of the orders Anseriformes (mainly ducks, geese and swans) and Charadriiformes
65 (mainly gulls, terns and waders) play a major role in the epidemiology of low pathogenic
66 avian influenza viruses (LPAIVs). LPAIVs are characterized into subtypes based on their
67 surface proteins hemagglutinin (HA, H1-H16) and neuraminidase (NA, N1-N9), e.g. H13N6.
68 Ducks play an important role in the epidemiology of most LPAIV subtypes. However, birds
69 of the order Charadriiforme*s*—in particular gulls— are the major reservoir for subtypes H13
70 and H16 (Table S1) (1-4). High prevalence of H13 and/or H16 LPAIVs has been observed in
71 juvenile gulls at breeding colony sites (5-7) and in adults during spring and/or fall migration
72 (8, 9). H13 and H16 viruses have a global distribution. Since first detection in 1977, H13
73 viruses have been detected in North America, South America, Europe, Asia, Africa and
74 Oceania. Since their first detection in 1975, H16 viruses have been detected in North
75 America, South America, Europe and Asia. The spatial isolation of host populations has
76 shaped LPAIV evolution and led to the independent circulation of different virus gene pools
77 between Western and Eastern hemispheres (10). Yet, some pelagic gull populations connect
78 multiple continents through seasonal migration and overlapping distributions and could
79 facilitate rapid and long-distance dispersal of LPAIV genomes (2, 9, 11-14). For instance,
80 great black-backed gulls (*Larus marinus*) migrate between Europe and the east coast of North
81 America, and LPAIVs consisting of both North American as well as Eurasian genes have
82 been isolated from this species (12). Upon intercontinental gene flow, i.e. the movement of
83 genes between the different continents, some LPAIV genes seem to have become established
84 in the population, e.g. H6 (15).

85 Influenza A viruses (IAV) belong to the family Orthomyxoviridae and are negative
86 sense single-stranded RNA viruses with a segmented genome. The genome consists of eight

87 segments encoding 12 proteins or more, including the surface proteins HA and NA. The HA
88 protein of IAV is a major determinant for virus binding to cells and subsequent cell entry and
89 for generation of IAV-specific antibodies, and thus subjected to strong selective pressure (16).
90 Indeed, in wild birds—in particular mallards (*Anas platyrhynchos*)—LPAIV infection
91 dynamics seem to be shaped between LPAIV subtypes partially by pre-existing homo- or
92 heterologous antibodies (17). Furthermore, within other host systems, evasion of IAV-specific
93 antibodies by IAVs—so called antigenic variation—has been described for seasonal human
94 IAVs (18, 19), swine IAVs (20-22), equine IAVs (23) and for highly pathogenic avian
95 influenza viruses (HPAIVs) that circulate in poultry (24, 25). Despite numerous studies on the
96 genetic variation of LPAIVs in wild birds, the antigenic variation within LPAIV subtypes that
97 circulate in wild birds is barely investigated (26, 27).
98 To better understand LPAIV epidemiology in gulls, we investigated the global
99 distribution of H13 and H16 LPAIVs and the antigenic variation of a representative subset of
100 H13 and H16 LPAIVs. Based on the sequencing of HA genes of 84 viruses, and
101 hemagglutination inhibition assays, we showed that intercontinental H13 and H16 gene flow
102 occurred frequently, and that H16 genetic lineages did not form antigenic clusters, suggesting
103 that clade-defining mutations were not in critical epitopes (i.e. part of the antigen that binds to
104 specific antibodies). In contrast, the H13 genetic clades partially corresponded with the
105 antigenic variation of H13 LPAIVs, suggesting part of the clade-defining mutations were in
106 critical epitopes.
107
108 **Results**
109
110 ***Phylogeographic structure and intercontinental gene flow***
111

112 Phylogenetic analyses supported that the H13 HA was structured in three major genetic
113 lineages (A-C; Figure 1, S1 and S2). The time to the most recent common ancestor (tMRCA)
114 of the H13 HA gene was dated in 1927 (± 95% HPD (highest posterior density): [1920-
115 1934]). The tMRCA of viruses of clade A (1963 [1958-1966]) was older than the ones of
116 clade B (1975 [1974-1976]) and C (1977 [1976-1978]). Our analyses support that the
117 geographic origin of H13 viruses of clade B and C could be North America and Europe,
118 respectively (posterior probabilities for the geographic origin of the most recent common
119 ancestor [MRCA]: 1 for clade B and 1 for clade C). For clade A, limited historical data of
120 viruses from different locations as well as low posterior probability (0.62) precludes a
121 conclusion on the geographic origin of the MRCA.

122 Since the first isolation of an H13 IAV from a gull in 1977, 20 potential events of
123 intercontinental gene flow were identified (indicated with 1-20 in Figure 1, S3 and Table 2).
124 Clade A supports the maintenance of H13 in European gulls, with evidence of multiple
125 introductions to North America and Asia (events #3, #5, #6, #7, and #10), and a reverse
126 introduction from North America to Asia (event #8). Clade C was also composed mainly of
127 viruses circulating in Europe, with evidence of multiple introductions to North America
128 (events #12, #15, #19) and Asia (events #13, #16, #17). The introduction of clade C H13 HA
129 in North America (event #19) was followed by an introduction to South America (event #20).
130 Evidence for intercontinental gene flow among North American H13 IAV occurred among
131 eastern and western North American isolates (event #3, #12, #15 and #19). Clade B was
132 composed almost exclusively of viruses circulating in North America, although one gene flow
133 event to South America occurred recently (event #11).

134 The H16 HA was structured in at least two major genetic lineages (Figure 2, S4 and
135 S5). The MCC tree was structured in three main clades (A-C, Figure S5), while the ML tree
136 provided support for only two main genetic clades (A and B/C merged, Figure S4). The

137 tMRCA of the H16 HA gene was dated in 1924 [1914-1932]. Clade A included only viruses
138 from Europe and was dated in 1977 [1975-1980]; clade B included only viruses from North
139 America with a time to the tMRCA estimated in 1969 [1967-1971]. Our analyses supported
140 that the geographic origin of clade A and B was Europe and North America, respectively
141 (posterior probabilities for the geographic origin of the MRCA: 0.99 for clade A, 1 for clade
142 B). The tMRCA of clade C was estimated 1965 [1962-1968]. Clade C may have arisen in
143 Europe (posterior probabilities for the geographic origin of the MRCA: 0.87) and consisted of
144 viruses of mixed origin, *i.e*. Europe, Asia and North America.

145 Since the first isolation of an H16 IAV from a black-legged kittiwake (*Rissa*
146 *tridactyla*) in 1975, ten intercontinental gene flow events were identified for viruses of clade
147 C (indicated with 1-10 in Figure 2, S6 and Table 3). As for the H13 subtype, strong support
148 for gene flow between Europe and North America was found, in particular from North-
149 Western European countries: Denmark to North-eastern America (Delaware, New Hampshire,
150 Quebec), and Iceland to Newfoundland (events #6 and #10). Evidence for intercontinental
151 gene flow among North American H16 IAV occurred among eastern and western North
152 American isolates (event #3, #6, #8 and #10). In particular, intercontinental gene flow #8
153 seems to have been maintained in North America after its initial introduction in 2006 [2005-
154 2006], for at least ten years, and may have replaced clade B of H16 HA (Figure 2).

155 High rates of nucleotide substitution obtained for the H13 HA genetic lineages were
156 consistent with those previously reported for H4, H6 and H7 subtypes circulating in wild
157 ducks (Table 4). However, the nucleotide substitution rate of clade B—that consists
158 exclusively of North American IAV—was lower than mean rates and HPD obtained for the
159 other two H13 clades. The mean d_N/d_S rate obtained for the three H13 genetic clades were
160 comparable to those previously reported for other subtypes and suggests that the HA was
161 under strong purifying selection (Table 4). Nonetheless, a slightly higher d_N/d_S rate obtained

162 for clade B and C as compared to other lineages suggests that they may be subjected to a more
163 neutral selection. The mean nucleotide substitution and d_N/d_S rates for the H16 gene were also
164 consistent with H13 HA as well as with H4, H6 and H7 subtypes from wild ducks. However,
165 H16 clade C (European mixed)– that consisted of viruses of a geographically more mixed
166 origin – had slightly lower nucleotide substitution rates and higher d_N/d_S rates than clade A
167 (European) and clade B (North American) (Table 4).
168
169 ***Antigenic diversity between H13 and H16 LPAIV***
170
171 As expected from two different HA subtypes, the H13 and H16 viruses formed two separate
172 antigenic variants. The H13 and H16 viruses were generally well separated, forming groups
173 on opposite sides of the antigenic map (Figure 3, Table 5). A total of nine amino acid
174 positions within/near the receptor binding site of the HA were identified that differed
175 consistently between H13 and H16 viruses (based on alignments of 338 H13 and 192 H16 HA
176 indicated in Table 6), of those, amino acid position 145 was located in the 130-loop, 200 and
177 208 in the 190-helix and 231 and 233 in the 220-loop of the receptor binding site of the HA
178 (HA numbering based on (28, 29). Of those, amino acid position 233 was listed previously as
179 being involved in differences in receptor-binding site between HAs originating from *Laridae*
180 and *Anatidae* (30). Additionally, the amino acid at position 196 differed between H13 (valine
181 [V]) and H16 (aspartic acid [D]) viruses; this position may contribute to receptor binding
182 specificity as identified previously based on crystal structures of H5 and H13 LPAIV (31).
183 Due to non-specific cross-reactivity, two H13 viruses (i.e. HEGU/AK/458/85 and
184 HEGU/AK/479/85) had unexpected high titers against H16 antisera (Table 5) and were
185 therefore positioned in the center of the map and served to pull H13 and H16 together.
186

187 ***Antigenic diversity among H13 LPAIV***

188

189 The representative H13 viruses formed at least two different antigenic variants (Figure 3,
190 Table 5). The viruses of H13 clades A and B were genetically distinct (Figure 1) but were
191 antigenically similar (Figure 3), based on the H13 clade A antisera cross-reacting with H13
192 clade B viruses and vice versa. In contrast, H13 clade C viruses reacted poorly—if at all—
193 with antisera that were raised against clade A and B viruses, and, conversely, antisera against
194 clade C viruses rarely reacted with substantial titers with viruses of clade A and B. Thus, H13
195 clade A/B and H13 clade C viruses formed two different antigenic variants. The antigenic
196 diversity of H13 clade A/B combined is about the same as the antigenic diversity of the H13
197 clade C. One H13 clade B virus, i.e. LAGU/DB/1370/86, could not be placed well in the map
198 due to HI titers of 40 or lower (Table 5).

199 To gain insight into the molecular basis of the antigenic variation between H13 clade
200 A/B and C, amino acids that differed consistently among the different clades of H13 viruses
201 were indicated (based on the alignment of 338 H13, Table 6). A total of four amino acid
202 positions within/near the receptor binding site of the HA were identified that differed
203 consistently for clade A, B and/or C. Of those, amino acids at positions 149 and 254 differed
204 consistently between clade A/B and C. Viruses belonging to clade C—except a single virus
205 from South America that had a arginine (R) at position 149—had a deletion at position 149
206 (previously identified using a smaller dataset as position 154 (12)), in contrast to viruses of
207 clade A or B that had an aspartic acid (D), glutamic acid (E), asparagine (N) or serine (S) at
208 this position. The correlation between the antigenic distance of H13 representative viruses
209 from A/gull/MD/704/1977 (H13N6) (clade A)—the first detected H13 virus—and the number
210 of HA1 amino acid substitutions from A/gull/MD/704/1977 was 0.87 and was statistically
211 significant ($P < 0.0001$, Pearson correlation).

212

213 ***Antigenic diversity among H16 LPAIV***

214

215 The representative H16 viruses formed at least one antigenic variant (Figure 3 and Table 5).
216 The genetically distinct H16 clades A, B and C did not form separate antigenic clusters in the
217 map, which reflects the raw HI data as there are no patterns for any of the four H16 antisera
218 tested that correspond to the genetic lineages. The antigenic diversity of the H16 viruses is
219 within eight antigenic units, with BHGU/NL/1/07 being on the edge of this antigenic space
220 (i.e. low titers to all sera). The antigenic diversity of H16 clade A/B/C is about the same as the
221 antigenic diversity of the H13 clade A/B combined and similar to the antigenic diversity of
222 the H13 clade C.
223 Though clade A, B and C did not form separate antigenic clusters in our analysis, amino acids
224 that differed consistently among the different clades of H16 viruses were indicated (based on
225 the alignment of 192 H16 HA, Table 6). A total of three amino acid positions within/near the
226 receptor binding site of the HA were identified that differed consistently among the three H16
227 clades and were not associated with antigenic variation. The correlation between the antigenic
228 distance of the representative viruses from A/Black-headed gull/TM/13/76 (H16N3) (clade
229 C)—one of the first detected H16 viruses—and the number of HA1 amino acid substitutions
230 from A/Black-headed gull/TM/13/76 was 0.67 and was statistically significant (P = 0.003,
231 Pearson correlation).

232

233 **Discussion**

234

235 We investigated the evolutionary history and intercontinental gene flow based on the
236 hemagglutinin (HA) gene of H13 and H16 LPAIV and selected representative viruses from

237 genetically distinct lineages to determine their antigenic properties by HI assays. H13 formed
238 at least three distinct genetic clades as suggested previously based on smaller datasets (9, 32-
239 35), while H16 formed at least two distinct genetic clades. Twenty and ten events of
240 intercontinental gene flow were identified for H13 and for H16 viruses, respectively. At least
241 two antigenic variants of H13 and at least one antigenic variant of H16 were identified. The
242 presence of different antigenic variants among viruses of a single LPAIV subtype is in
243 contrast to previous findings based on antigenic characterization of LPAIV H3 (26), and
244 implies that antigenic variation within LPAIV subtypes occurs.

245 The frequency of intercontinental gene flow of the HA gene of H13 and H16 viruses
246 was similar to the HA gene of H6 viruses, but lower than for internal genes (2, 27, 36, 37).
247 Previously, intercontinental gene flow has been described extensively for the H6 HA genes,
248 while no intercontinental gene flow was detected for the H4 and H7 subtypes (15, 38). For the
249 H6 subtype, gene flow has been described ten times with four established genes during a
250 period of 31 years (1975-2006; (15)). Also, evidence for intercontinental gene flow among
251 North American H13 and H16 genes occurred among eastern and western North American
252 LPAIVs in contrast to eastern North American LPAIVs only as reported previously (39).
253 Given the relatively high number of intercontinental flow of IAV internal genes by shorebirds
254 and gulls (2, 27, 36, 37), one may have expected a higher gene flow of gull-associated H13
255 and H16 HA genes, compared to e.g. H6. However, a higher intercontinental gene flow only
256 was apparent with H13 (i.e. 20 events during a period of 35 years). This may suggest i)
257 broader host range, host population size and/or host distribution of H13 than H16, and/or ii)
258 local H13-specific herd-immunity is lower than H16-specific herd immunity and therefore
259 less limiting establishment opportunities in host populations of H13, and/or iii) higher
260 environmental survival of H13 than of H16, and/or iv) introduced H13 HA genes may be less
261 affected by strong subtype-dependant competition with endemic HA genes (e.g. with respect

262 to linkage to NS1 and NP as these contain most gull-specific features (33)) than introduced
263 H16 genes. Interestingly, no H13 or H16 gene flow was described from Asia to Europe,
264 which is in contrast to e.g. HPAIV H5 viruses that have been introduced from Asia to Europe
265 several times (40, 41). The relatively low frequency of detection of intercontinental gene flow
266 of H13 or H16 genes out of North America and in particular Asia, relative to Europe, may be
267 due to a bias in IAV surveillance and sequencing (i.e. number of available IAV sequences
268 from gulls isolated in Europe is higher than from North America and in particular Asia).
269 Antigenic diversity of LPAIV depends partially on the host population size and
270 structure. In this study, both H13 and H16 LPAIV formed at least three or two distinct genetic
271 clades respectively that did not or only partially corresponded with antigenic clusters. The
272 H16 genetic clades did not form antigenic clusters, suggesting that clade-defining mutations
273 were not in critical epitopes. In contrast, the H13 genetic clades partially corresponded with
274 the antigenic variation of H13 LPAIV, suggesting that part of the clade-defining mutations
275 were in critical epitopes. Also, given that the H13 antigenic space is larger than the antigenic
276 space covered by H16 viruses, the host population of H13 may be larger and more widely
277 distributed than the host population of H16 LPAIV, facilitating the circulation of more than
278 one antigenic variant of a single LPAIV subtype. Strong genetic and antigenic divergence
279 between two co-circulating lineages could be the product of a very large host meta-population
280 size and relatively rare cross-species transmission rate (42). Globally, viruses of the H13
281 subtype seem to be more common than viruses of the H16 subtype (2, 4), which is consistent
282 with the finding that H13 LPAIV consists of multiple antigenic variants. Besides increased
283 host population size and host distribution, prolonged virus survival may shape LPAIV
284 epidemiology and evolution. Antigenic diversity within H13 LPAIV may be shaped by amino
285 acid substitutions near the receptor binding site of the HA protein. In this study, we found
286 evidence that amino acids or deletions at positions 149 and 254 of the HA protein may be

287 involved in antigenic diversity among H13 strains. In addition, position 149 could be involved
288 in H16 LPAIV antigenic diversity as all H16 viruses had a deletion at this position and H16
289 clade A, B and C were antigenically similar.

290 Co-circulating and newly introduced H13 or H16 LPAIV can be either antigenically
291 similar or antigenically different. In the Northern hemisphere, H13 and H16 IAV subtypes
292 circulate most extensively on breeding colonies in hatch-year birds at the end of summer and
293 early fall (5-7). In black-headed gulls (which in Europe are one of the main host for H13 and
294 H16 LPAIV), infection with H13 or H16 result in strong protection against reinfection with
295 the same virus, however susceptibility to infection with the other subtype or with another
296 strain of the same subtype is unknown (43, 44). Our findings support the independent long-
297 term maintenance and co-circulation of at least two genetically distinct lineages of H13 and of
298 H16 in Eurasia. This pattern is similar to the one that has been described for the H3 IAV
299 subtype in ducks in North America (42). Our analysis showed that these genetically distinct
300 co-circulating lineages may belong to the same antigenic variant. Here, we found evidence
301 that genetically distinct co-circulating H13 or H16 LPAIV on a black-headed gull breeding
302 colony site in the Netherlands may be either antigenically different (e.g. H13 clade A virus
303 A/BHGU/NL/7/2009 (H13N2) and H13 clade C virus A/BHGU/NL/20/2009 (H13N2) or
304 antigenically similar (e.g. H16 clade A A/BHGU/NL/10/2009 (H16N3) and
305 A/BHGU/NL/21/2009 (H16N3) and H16 clade C A/BHGU/NL/26/2009 (H16N3). Similar,
306 intercontinental gene flow occurred with HA genes that were antigenically similar to local
307 circulating viruses (i.e. H16 clade C viruses that were genetically closely related to
308 SB/DB/172/06 and SB/DB/195/06 versus local circulating H16 clade B viruses), and HA
309 genes that were antigenically different from local circulating viruses (i.e. H13 clade C viruses,
310 genetically closely related to LAGU/NJ/AI08-0714/08 versus local circulating H13 clade B
311 viruses.

312 Antigenic variation within a LPAIV subtype at the clade level (i.e. H13 clade A/B
313 combined versus H13 clade C) was described here, yet less is known about antigenic variation
314 within genetic clades of H13, H16 or other LPAIV subtypes. For H13, genetic diversity
315 within clades seemed stable—e.g. viruses of clade A, B or C, collected over three decades
316 were antigenically closely related—suggesting no major genetic differences; this is in contrast
317 to the few mutations needed for antigenic change in seasonal human IAV. Similarly, a study
318 on antigenic variation of H3 LPAIV isolated in North America suggested that genetically
319 diverse viruses were antigenically stable (26). Major antigenic changes in seasonal human
320 IAV were due to amino acid substitutions immediately adjacent to the receptor binding site
321 (18); this could potentially also explain antigenic variation between antigenically different
322 viruses of H13 clade A/B combined and clade C (i.e. amino acid positions 149 of the HA).
323 Future work on antigenic variation of LPAIV should include within clade genetic and
324 antigenic variation.
325
326 **Materials and Methods**
327
328 **Viruses.** The HA sequences of H13 (n=64) and H16 (n=20) viruses isolated from wild birds
329 in North America (n=39 and n=5, respectively) and Europe (n=25 and n=15, respectively)
330 between 1976 and 2010 were determined at the University of Minnesota (Saint Paul,
331 Minnesota, USA) and at the Department of Viroscience of the Erasmus Medical Center
332 (Rotterdam, the Netherlands). Details on virus isolates including GenBank accession numbers
333 are summarized in Table S2 and S3; details related to the Sanger sequencing methodology are
334 available upon request. The HA sequences were supplemented with full-length nucleotide
335 sequences of the HA gene of H13 and H16 viruses isolated from wild birds between 1975 and
336 2017 and downloaded from GenBank (https://www.ncbi.nlm.nih.gov). The full dataset

337 included sequences of H13 (n=519) and H16 (n=276) HA genes and was biased towards virus
338 strains collected since 2000 due to increased surveillance and sequencing since 2000.
339 Of this full dataset, viruses representing the genetically distinct clades were selected (n=44;
340 H13 clade A, B, C and H16 clade A, B, C; see the Results section for clade definition) to
341 investigate the antigenic diversity of H13 and H16 viruses. Of those viruses, viruses that were
342 genetically most divergent were selected (n=10) to generate ferret antisera (Table 1). The
343 antigenic properties of all representative viruses (n=44) were analysed in hemagglutination
344 inhibition (HI) assays using the panel of ten ferret antisera.
345
346 **Genetic analyses.** The nucleotide sequences of the coding region of H13 and H16 HA were
347 aligned with the program CLC 8.0 (CLC bio, Aarhus, Denmark). Neighbor-Joining trees were
348 then generated, with 1000 bootstraps, in order to assess the overall genetic structure of the
349 H13 (n=519) and H16 (n=276) HA sequences. To lower the bias in species and geography
350 (e.g. black-headed gulls (*Chroicocephalus ridibundus*) from the Netherlands and glaucous-
351 winged gulls (*Larus glaucescens*) from Alaska), duplicate sequences (i.e. identical sequences
352 of the same host species, location and date) were identified with Mothur 1.39.5 (45) and
353 removed, resulting in final alignments of H13 (n=338) and H16 (n=192) HA. To identify the
354 genetic structure of H13 and H16 virus subtypes Maximum-likelihood trees with 1000
355 bootstraps were generated with the software PhyML 3.1 (46). The general time reversible
356 (GTR) evolutionary model, an estimation of the proportion of invariable sites (I) and of the
357 nucleotide heterogeneity of substitution rate (α) was used as selected by Model Generator
358 0.85 (47). To investigate the evolutionary history of H13 and H16 virus subtypes Bayesian
359 Markov Chain Monte Carlo coalescent analyses were performed. The temporal structure of
360 the dataset was assessed with the program TempEst 1.5.3 (48). Both datasets showed a
361 positive correlation between genetic divergence and sampling time and appear to be suitable

362 for phylogenetic molecular clock analyses. Time to the most recent common ancestors
363 (MRCA) as well as geographic ancestral states (i.e. continent), and their associated posterior
364 probabilities were obtained based on the method described by Lemey et al. with the program
365 BEAST 1.10.1 (49, 50). A strict molecular clock model was selected as relaxed clock models
366 (uncorrelated exponential and uncorrelated lognormal) resulted in low effective sample sizes
367 (ESS < 200) in spite of high chain length (>200 million states). In all simulations a Bayesian
368 skyline coalescent tree prior (51) was selected. The Shapiro-Rambaut-Drummond-2006
369 nucleotide substitution model was selected (52), and has been used in population dynamic
370 studies of other IAV subtypes (15, 38, 42, 53). Overall, a similar methodology was used as in
371 previous studies on IAV evolutionary dynamics of subtypes H4, H6 and H7 (15, 38, 54).
372 Analyses were performed with two independent chain lengths of 100 million generations
373 sampled every 1000 iterations; the first 10% of trees were discarded as burn-in. Substitutions
374 rates based on independent analyses of the major H13 and H16 clades were obtained using the
375 program BEAST 1.10.1. Nonsynonymous substitutions (d_N) and synonymous substitutions
376 (d_S) rates were obtained using the single likelihood ancestor counting method implemented in
377 HyPhy (55). Computations were performed with the Datamonkey webserver (56, 57).
378
379 **Antisera.** Post-infection antisera were prepared upon nasal inoculation of ferrets (> 1 year of
380 age, male, two ferrets per virus) with virus (cultured on embryonated chicken eggs, per ferret
381 10^6 - 10^7 median egg infectious dose (EID_{50})/100 µl) and blood collection by exsanguination
382 14 days later. An overview of antisera used in this study is provided in Table 1. Antisera were
383 pre-treated overnight at 37ºC with receptor-destroying enzyme (*Vibrio cholerae*
384 neuraminidase), followed by inactivation for 1 hr at 56ºC before use in HI assays.
385
386 **Antigenic analyses.** HI assays were performed according to standard procedures (58). The HI

387 titer is expressed as the reciprocal value of the highest serum dilution that completely
388 inhibited hemagglutination. To investigate antigenic variation among and within H13 and
389 H16 viruses, antigenic cartography methods were used as described previously (19). Briefly,
390 antigenic cartography is a method to analyse and visualize HI assay data. The titers in an HI
391 table can be thought of as specifying target distances between antigens and antisera. In an
392 antigenic map, the distance between antigen point A and antiserum point S corresponds to the
393 difference between the $\log_2$ value of the maximum observed titer to antiserum S from any
394 antigen and the titer of antigen A to antiserum S. Modified multidimensional scaling methods
395 are used to arrange the antiserum and antigen points in an antigenic map to best satisfy the
396 target distances specified by the HI data (18). Because antigens are tested against multiple
397 antisera, and antisera are tested against multiple antigens, many measurements can be used to
398 determine the position of the antigens and antisera in an antigenic map, thus improving the
399 resolution of the HI data.
400
401 **Ethics statement.** This study was approved by the independent animal experimentation
402 ethical review committee Stichting DEC consult (Erasmus MC permit 122-98-01, 122-08-04
403 and 15-340-03) and was performed under animal biosafety level 2 (ABSL-2) conditions.
404 Animal welfare was monitored daily, and all animal handling was performed under light
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406
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606

607 **Figure legends**

608 **Figure 1.** Maximum clade credibility (MCC) trees for influenza A virus H13 hemagglutinin
609 subtype (n= 338). Branches were colored according to most probable geographic origin (red:
610 North America; orange: South America; dark blue: Europe; light blue: Asia; green: Oceania;
611 gray: not identified). Black node bars represent the 95% highest posterior densities for times
612 of the common ancestors. Numbers highlight intercontinental gene flow events as detailed in
613 Table 2 and Figure S3. Virus strain names and posterior probabilities are detailed in Figure

614 S2.

615

616 **Figure 2.** Maximum clade credibility (MCC) trees for influenza A virus H16 hemagglutinin
617 subtype (n=192). Branches were colored according to most probable geographic origin (red:
618 North America; orange: South America; dark blue: Europe; light blue: Asia; green: Oceania;
619 gray: not identified). Black node bars represent the 95% highest posterior densities for times
620 of the common ancestors. Numbers highlight intercontinental gene flow events as detailed in
621 Table 3 and Figure S6. Virus strain names and posterior probabilities are presented in Figure
622 S5.

623

624 **Figure 3.** Antigenic map of H13 and H16 influenza A viruses (n=44). Different subtypes and
625 genetic clades are indicated with colors (yellow: H13 clade A; orange: H13 clade B; red: H13
626 clade C; blue: H16 clade A; purple: H16 clade B; green: H16 clade C). White circles indicate
627 the antisera. Respective virus strains are abbreviated; the full name can be found in Table 5.
628 Asterices indicates antigens BHGU/NL/20/09, BHGU/SE/1/06, BHGU/SE/1/03,
629 GBBG/AK/1421/79, BHGU/NL/1/07, HEGU/NY/AI00-532/00 and LAGU/NJ/AI08-0714/08
630 that had only two numerical HI titers to the tested sera and hence their placement in the map
631 is not robust. In this map the distance between the points represents antigenic distance as
632 measured by the hemagglutination inhibition (HI) assay in which the distances between
633 antigens and antisera are inversely related to the log2 HI titer. Each square in the grid of the
634 antigenic map equals a two-fold difference in the HI assay.

635

636 **Tables**

637 **Table 1.** Representative viruses selected to generate ferret antisera used to map the antigenic
638 diversity of H13 and H16 influenza A viruses

Subtype	Clade	Virus strain name
H13	A	A/Gull/Maryland/704/1977 (H13N6)
	A	A/Black-headed gull/Netherlands/2/2007 (H13N6)
	B	A/Ring-billed gull/Georgia/AI00-2658/2000 (H13N6)
	B	A/Gull/Minnesota/1352/1981 (H13N6)
	C	A/Laughing gull/ New Jersey/AI08-0714/ 2008 (H13N9)
	C	A/Great black-headed gull/Astrakhan/1420/1979 (H13N2)
H16	A	A/Black-headed gull/Sweden/2/1999 (H16N3)
	B	A/Herring gull/New York/AI00-532/2000 (H16N3)
	C	A/Black-headed gull/Turkmenistan/13/1976 (H16N3)
	C	A/Black-headed gull/Sweden/5/1999 (H16N3)

Table 2. Intercontinental gene flow events for influenza A virus H13 hemagglutinin. MRCA: Most Recent Common Ancestor. HPD: Higher Posterior Density. Event # corresponds to the numbers indicated in Figure 1 and S3

H13 Clade	**Event #**	**Time of the MRCA ± 95% HPD**	**Geographic origin of the MRCA (posterior)**	**Location of introduction**
A	1	1963 [1958-1966]	North America (0.62)	Oceania

	2	1974 [1972-1975]	North America (0.73)	Europe
	3	1988 [1987-1989]	Europe (1)	North America
	4	1990 [1988-1991]	Europe (0.82)	South America
	5	1996 [1995-1997]	Europe (0.75)	Asia
	6	2003 [2003-2004]	Europe (1)	Asia
	7	2005 [2004-2005]	Asia (0.48)	North America
	8	2009 (2009-2010]	North America (0.9)	Asia
	9	2006 [2006-2007]	Europe (0.96)	Asia
	10	2011 [2010-2011]	Europe (1)	Asia
B	11	2013 [2012-2014]	North America (0.96)	South America
C	12	1987 [1985-1988]	Europe (0.99)	North America

13	2002 [2002-2003]	Europe (1)	Asia
14	2005 [2004-2005]	Asia (0.55)	North America
15	2010 [2009-2010]	Europe (1)	North America
16	2004 [2003-2005]	Europe (0.97)	Asia
17	2013 [2013-2014]	Europe (0.99)	Asia
18	2014 [2013-2014]	North America (0.39)	Asia
19	2011 [2010-2011]	Europe (0.99)	North America
20	2012 [2011-2012)	North America (0.94)	South America

Table 3. Intercontinental gene flow events for influenza A virus H16 hemagglutinin. MRCA: Most Recent Common Ancestor. HPD: Higher Posterior Density. Event # corresponds to the numbers indicated in Figure 2 and S6

H16 Clade	Event #	Time of the MRCA ± 95% HPD	Geographic origin of the MRCA (posterior)	Location of introduction
C	1	1971 [1968-1972]	Europe (0.97)	Asia
	2	1976 [1976-1976]	Asia (0.71)	Europe
	3	1976 [1972-1980]	Europe (0.86)	North America
	4	1999 [1999-1999]	Europe (1)	Asia
	5	2003 [2002-2004]	Europe (1)	Asia
	6	1999 [1998-2000]	Europe (0.99)	North America
	7	2008 [2007-2009]	Europe (0.99)	Asia
	8	2006 [2005-2006]	Europe (0.97)	North America
	9	2006 [2006-2007]	North America (0.55)	South America
	10	2008 [2007-2009]	Europe (0.63)	North America

649

650 **Table 4.** Molecular evolution of the HA gene of influenza A virus subtypes H13 and H16

Genetic lineage	N[1]	Time period[2]	Substitution rate[3]		d_N/d_S
			Mean	95% HPD	Mean
H13	338	40	3.8	3.6-4.1	0.13
H13 - A	54	39	3.8	2.3-4.9	0.09
H13 - B	76	39	0.8	0.6-1.0	0.18
H13 - C	208	37	5.5	5.0-6.0	0.16
H16	192	41	3.1	2.8-3.4	0.09
H16 - A	56	33	4.5	3.9-5.2	0.10
H16 - B	19	35	4.6	3.9-5.2	0.06
H16 - C	117	40	1.5	1.2-1.8	0.11

651 [1] number of nucleotide sequences included in the analysis; [2] in years; [3] per 10^{-3} substitution / site /
652 year; HPD: highest posterior density.

653

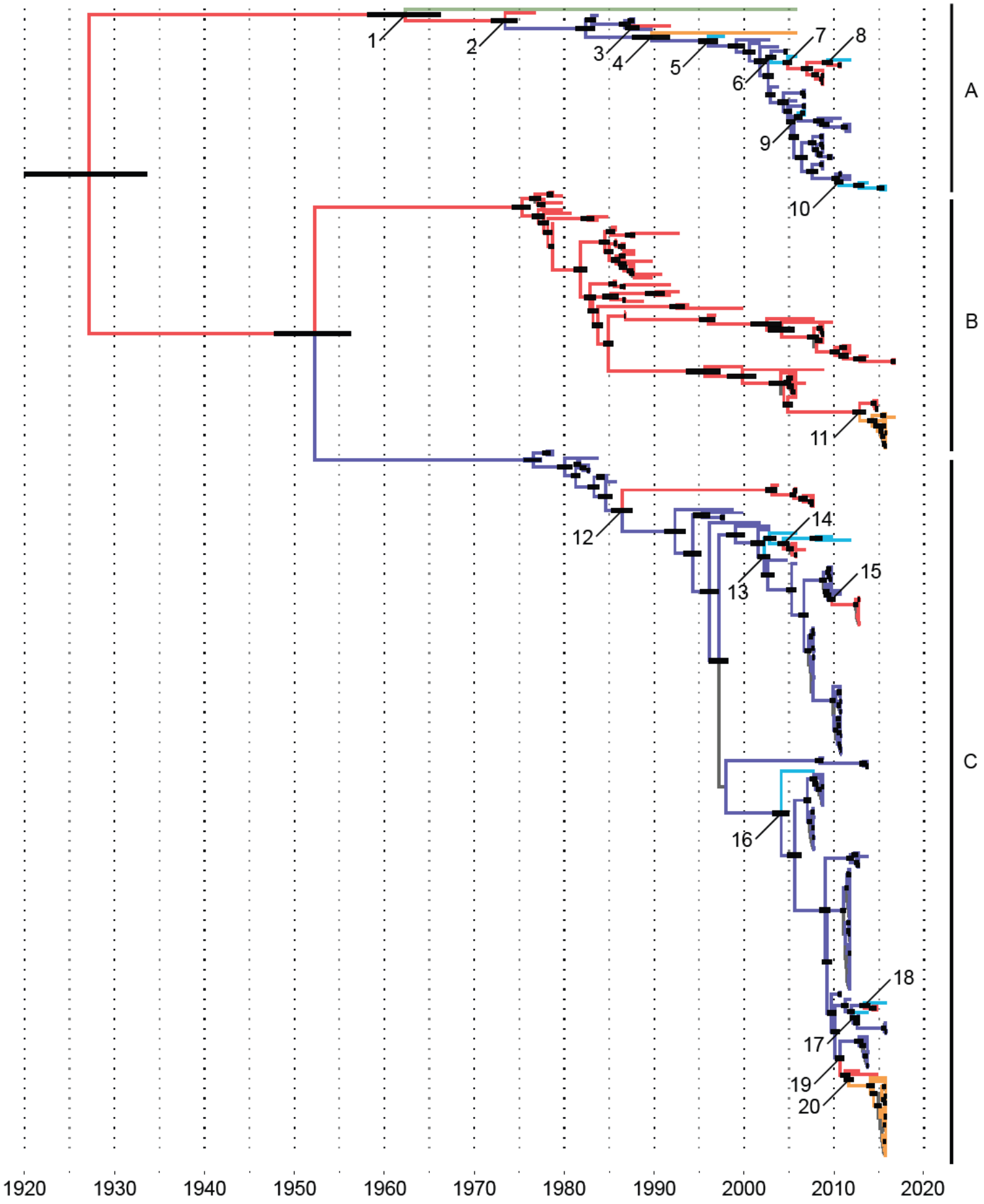
654 **Table 5.** Hemagglutinin inhibition data of H13 and H16 influenza A viruses (n=44)

Suptype				H13						H16			
Clade				A	A	B	B	C	C	A	B	C	C
	Virus name	Subtype	Virus abbreviation	BHGU/NL/2/07	GULL/ML/704/77	GULL/MN/1352/81	RBGU/GE/AI00-2658/00	GBBG/AK/1420/79	LAGU/NJ/AI08-714/08	BHGU/SE/2/99	HEGU/NY/AI0-532/00	BHGU/SE/5/99	BHGU/TM/13/76
H13 / A	A/Black-headed gull/Netherlands/2/07	H13N6	BHGU/NL/2/07	**320**	280	80	<10	20	<10	<10	<10	<10	25
	A/Black-headed gull/Netherlands/4/07	H13N6	BHGU/NL/4/07	1280	400	320	<10	35	<10	<10	<10	10	40
	A/Black-headed gull/Netherlands/7/09	H13N2	BHGU/NL/7/09	10	160	<10	<10	<10	<10	10	<10	<10	15
	A/Black-headed gull/Sweden/10/05	H13N6	BHGU/SE/10/05	240	320	40	<10	10	<10	<10	<10	<10	15
	A/Great-black headed gull/Sweden/1/03	H13N6	GBBG/SE/1/03	80	240	20	<10	<10	<10	<10	<10	<10	<10
	A/gull/ML/704/77	H13N6	GULL/ML/704/77	40	**240**	20	<10	<20	<10	<10	<10	<10	<10
H13 / B	A/gull/MN/1352/81	H13N6	GULL/MN/1352/81	120	160	320	<10	20	<10	<10	<10	<10	<10
	A/gull/NJ/34/92	H13N6	GULL/NJ/34/92	80	240	80	<10	240	<10	<10	<10	<10	<10
	A/Herring gull/DB/13/90	H13N2	HEGU/DB/13/90	40	140	140	10	25	<10	<10	<10	<10	<10
	A/Laughing gull/DB/1370/86	H13N2	LAGU/DB/1370/86	10	40	<10	10	40	<10	<10	<10	<10	<10
	A/ring-billed gull/GE/AI00-2658/00	H13N6	RBGU/GE/AI00-2658/00	10	60	40	**640**	15	<10	<10	<10	<10	<10

	A/ring-billed gull/MN/AI10-1708/10	H13N6	RBGU/MN/AI10-1708/10	80	200	120	10	10	<10	<10	<10	<10	<10
H13 / C	A/Black-headed gull/Netherlands/1/00	H13N8	BHGU/NL/1/00	35	<10	<10	<10	1280	120	<10	30	<10	30
	A/Black-headed gull/Netherlands/20/09	H13N2	BHGU/NL/20/09	<10	<10	<10	<10	280	<10	<10	<10	<10	35
	A/Black-headed gull/Netherlands/4/08	H13N8	BHGU/NL/4/08	<10	<10	<10	<10	140	80	<10	<10	<10	25
	A/Black-headed gull/Sweden/1/03	H13N8	BHGU/SE/1/03	<10	<10	<10	<10	560	40	<10	<10	<10	<10
	A/Black-headed gull/Sweden/1/06	H13N8	BHGU/SE/1/06	<10	<10	<10	<10	120	<10	<10	<10	<10	<10
	A/Black-headed gull/Sweden/1/99	H13N6	BHGU/SE/1/99	10	<10	10	30	160	<10	<10	<10	<10	10
	A/Black-headed gull/Sweden/2/03	H13N8	BHGU/SE/2/03	<10	<10	<10	<10	200	50	<10	<10	<10	10
	A/Great-black headed gull/AK/1420/79	H13N2	GBBG/AK/1420/79	10	35	10	<10	**2720**	160	10	<10	35	25
	A/Great-black headed gull/AK/1421/79	H13N2	GBBG/AK/1421/79	<10	<10	<10	<10	140	80	<10	<10	<10	<10
	A/Great-black headed gull/AK/591/82	H13N2	GBBG/AK/591/82	<10	40	<10	<10	480	100	<10	<10	40	80
	A/Great-black headed gull/GJ/76/83	H13N2	GBBG/GJ/76/83	<10	<10	<10	<10	320	80	<10	<10	<10	30
	A/Herring gull/AK/458/85	H13N6	HEGU/AK/458/85	30	20	<10	<10	1920	480	70	<10	80	80
	A/Herring gull/AK/479/85	H13N6	HEGU/AK/479/85	140	35	10	<10	1920	640	280	120	280	120
	A/Laughing gull/NJ/AI08-714/08	H13N9	LAGU/NJ/AI08-714/08	<10	<10	<10	<10	320	**560**	<10	<10	<10	<10
H16 / A	A/Black-headed gull/Netherlands/5/07	H16N3	BHGU/NL/5/07	35	25	<10	<10	140	<10	960	160	320	640
	A/Black-headed gull/Netherlands/1/07	H16N3	BHGU/NL/1/07	<10	<10	<10	<10	<10	<10	80	<10	<10	40
	A/Black-headed gull/Netherlands/10/09	H16N3	BHGU/NL/10/09	20	80	<10	<10	280	15	1280	160	640	640
	A/Black-headed gull/Netherlands/21/09	H16N3	BHGU/NL/21/09	70	200	20	<10	240	<10	480	<10	240	280
	A/Black-headed gull/Netherlands/3/07	H16N3	BHGU/NL/3/07	100	90	20	<10	100	<10	120	140	60	120
	A/Black-headed gull/Sweden/2/99	H16N3	BHGU/SE/2/99	10	<10	<10	<10	10	<10	**960**	80	35	380
	A/Black-headed gull/Sweden/8/05	H16N3	BHGU/SE/8/05	<10	<10	<10	<10	10	<10	1280	<10	30	140
H16 / B	A/Herring gull/DB/2617/87	H16N3	HEGU/DB/2617/87	<10	<10	<10	<10	<10	<10	<10	120	20	1600
	A/Herring gull/NY/AI0-532/00	H16N3	HEGU/NY/AI0-532/00	<10	<10	<10	<10	<10	<10	<10	**320**	<10	320
	A/Laughing gull/DB/2839/87	H16N3	LAGU/DB/2839/87	<10	<10	<10	<10	<10	<10	160	80	20	1920
H16 / C	A/Black-headed gull/Netherlands/26/09	H16N3	BHGU/NL/26/09	10	25	<10	<10	20	<10	30	80	20	1280
	A/Black-headed gull/Sweden/5/99	H16N3	BHGU/SE/5/99	10	<10	<10	<10	70	<10	560	30	**1600**	400
	A/Black-headed gull/TM/13/76	H16N3	BHGU/TM/13/76	25	30	<10	<10	27,5	<10	50	320	100	**4800**
	A/environment/Sweden/2/05	H16N3	ENV/SE/2/05	20	30	10	<10	140	30	960	320	1280	640
	A/Little tern/Sweden/1/05	H16N3	LITE/SE/1/05	<10	15	<10	<10	15	<10	10	30	20	1280
	A/shorebird/DB/172/05	H16N3	SB/DB/172/05	<10	<10	<10	<10	30	<10	240	60	200	1280
	A/shorebird/DB/195/06	H16N3	SB/DB/195/06	<10	<10	<10	<10	<10	<10	<10	30	20	560
	A/Slender-billed gull/AK/28/76	H16N3	SBGU/AK/28/76	20	140	10	<10	50	<10	80	160	100	1280

Table 6. Amino acid differences within/near the receptor binding site of the HA protein among H13 and H16 subtypes and clades, based on the HA gene of H13 (n=338) and H16 (n=192) LPAIVs, including the 130-loop (position 136-147 according to Burke & Smith 2014), 190-helix (200-208) and 220-loop (230-240). DEL, deletion of amino acid.

Amino acid position Clade	**139**	**142**	**145**	**149**	**166**	**176**	**177**	**196**	**198**	**200**	**208**	**217**	**218**	**224**	**231**	**233**
H13 A	D	A,T,S	A	D,E,N,S	K,Q	K	T	V,L	V	E	S,G	K	S,L	K	P	Y
H13 B	D	A,T,S	A	D,N,S	K,R	G,R	T	V,I	T,A	E	S,G	S,R,N,H	S,L	K,N	P,L	Y,Q
H13 C	D	V,A	A	DEL,R	K,R,S	G,R	T,A,V	V,I	T,A,E	E	D,N,S	S,R,G	S,T	N,T,K	P	Y
H16 A	E	T	S	DEL	L	G	E	D	E	T	K	K	E	E	I	D
H16 B	D	V	S	DEL	DEL	G	D	D	E,?	T,V	K	K,E	E	E	I	D,E,N
H16 C	D	V,A	S	DEL	K,DEL	G	E,D	D	E	T	K	K	E	E	I,V	D,N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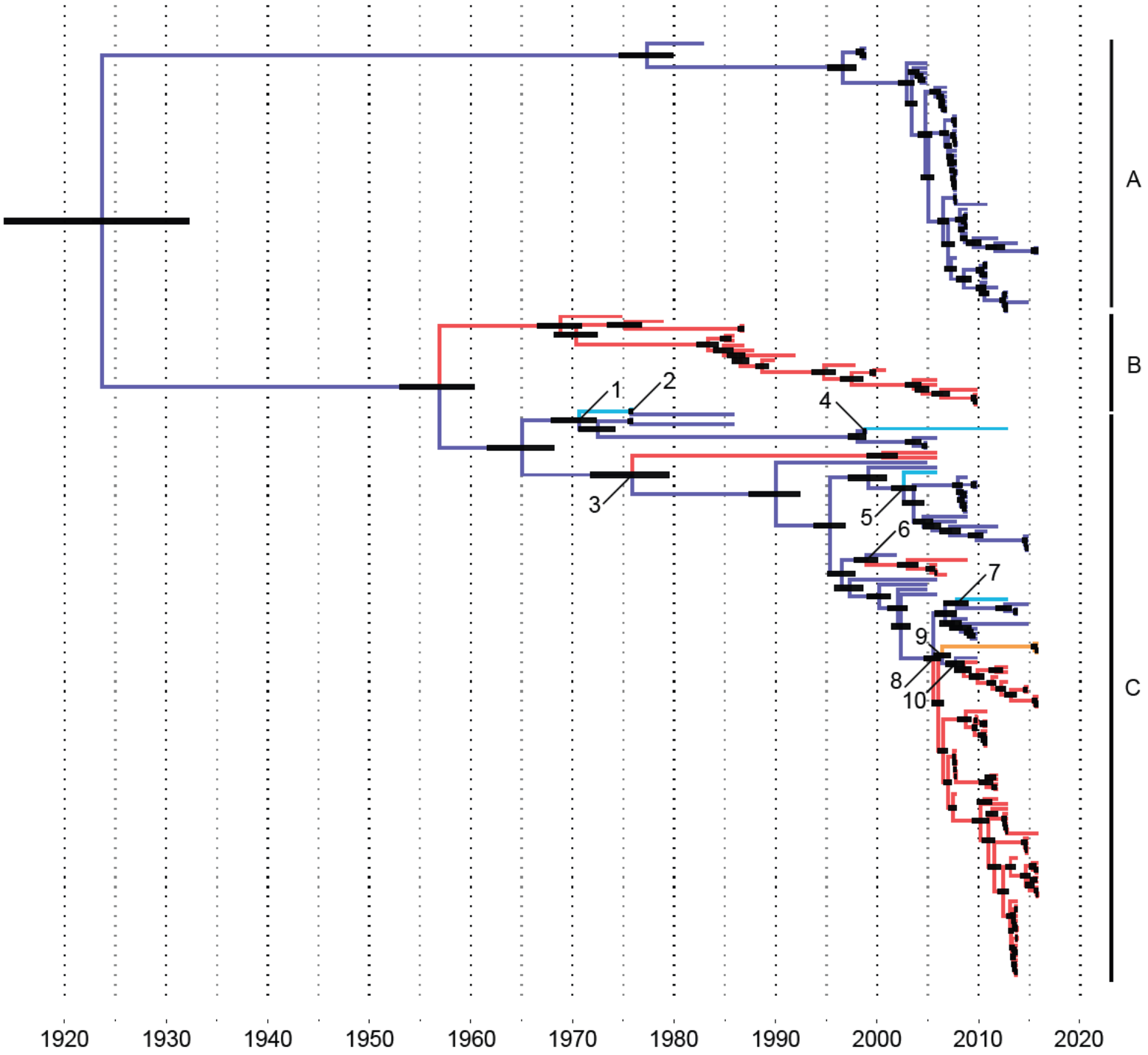
A
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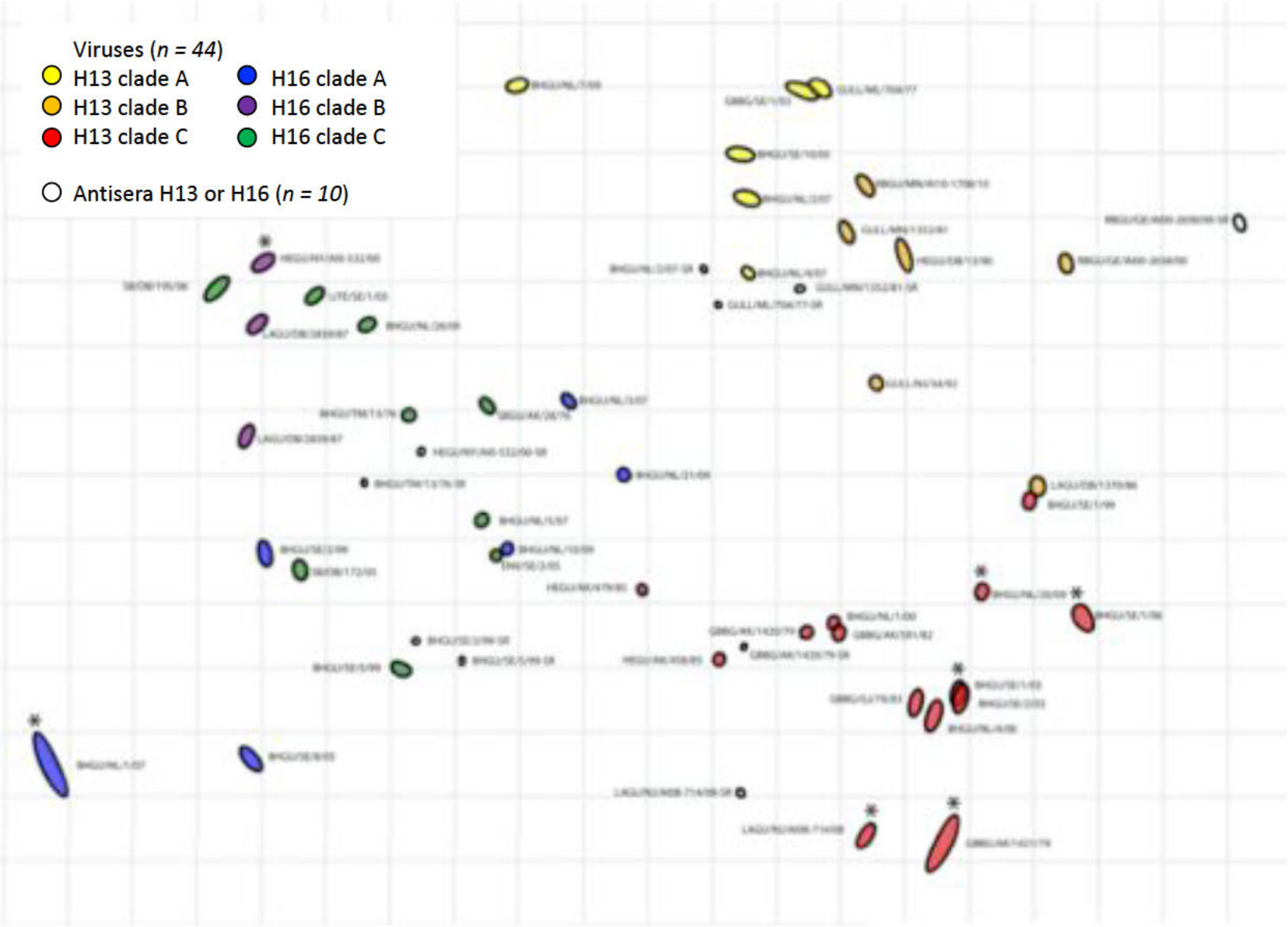
Viruses (*n = 44*)
H13 clade A
H13 clade B
H13 clade C
H16 clade A
H16 clade B
H16 clade C
Antisera H13 or H16 (*n = 10*)

Supplementary Material of

"Phylogeography and antigenic diversity of low pathogenic avian influenza H13 and H16 viruses"

Figure S1. Maximum-Likelihood tree obtained with H13 HA sequences (n=338) and 1000 bootstraps. Virus names in bold were sequenced as part of this study. Those highlighted in red were used for the antigenic analyses. Only bootstrap values higher than 50 are indicated.

Figure S2. Maximum clade credibility tree for influenza A virus H13 hemagglutinin subtype (n=338). Posterior probabilities are reported when higher than 0.5. Virus names in bold were sequenced as part of this study. Those highlighted in red were used for the antigenic analyses. Node bars indicate 95% highest posterior density for times of the most recent common ancestors. Scale bar indicates 10 years.

Figure S3. World map indicating intercontinental gene flow of influenza A virus H13 hemagglutinin in time. Numbers highlight intercontinental gene flow events as detailed in Table 2 and Figure 1. Arrows indicate direction of gene flow. Colors indicate time interval between the most recent common ancestor (MRCA) and the detected H13 LPAIV. Continuous line: posterior probability of >0.95; dotted line: posterior probability of ≤0.95.

Figure S4. Maximum-Likelihood tree obtained with H16 HA sequences (n=192) and 1000 bootstraps. Virus names in bold were sequenced as part of this study. Those highlighted in red were used for the antigenic analyses. Only bootstrap values higher than 90 are indicated.

Figure S5. Maximum clade credibility tree for influenza A virus H16 hemagglutinin subtype

(n=192). Posterior probabilities are reported when higher than 0.5. Virus names in bold were sequenced as part of this study. Those highlighted in red were used for the antigenic analyses. Node bars indicate 95% highest posterior density for times of the most recent common ancestors. Scale bar indicates 10 years.

Figure S6. World map indicating intercontinental gene flow of influenza A virus H16 hemagglutinin in time. Numbers highlight intercontinental gene flow events as detailed in Table 3 and Figure 2. Arrows indicate direction of gene flow. Colors indicate time interval between the most recent common ancestor (MRCA) and the detected H16 LPAIV. Continuous line: posterior probability of >0.95; dotted line: posterior probability of ≤0.95.

Table S1. Distribution of influenza A virus subtypes among gull species in Eurasia and America based on the Influenza Research Database (IRD, https://www.fludb.org) (d.d. 20-Dec-2019). Subtype not detected (-)

Table S2. List of H13 HA influenza A viruses (n=519) and corresponding accession number included in the study. Virus names in bold were sequenced as part of this study.

Table S3. List of H16 HA influenza A viruses (n=276) and corresponding accession numbers included in the study. Virus names in bold were sequenced as part of this study.

Figure S1


A/SilverGull/Tasmania/62/2006 H13N6 CY094903
A/Gull/MD/704/77 H13N6 KF612959
A/Gull/Astrakhan/226/1984 H13N6 EU835895
A/Larus ichthyaetus/Astrakhan/10/1988 H13N6 EU564106
A/Larus ichthyaetus/Astrakhan/44/1988 H13N6 EU564115
A/Gull/BD/44036/92 H13N6 KF612960
A/Gull/Astrakhan/998/1990 H13N6 EU835896
A/Kelp Gull/Argentina/LDC4/2006 H13N9 EU523136
A/Duck/Siberia/272/1998 H13N6 AB284988
A/Great black-backed Gull/Sweden/1/2003 H13N6 KR087577
A/Gull/Astrakhan/3453/2002 H13N6 EU835897
A/Great Black-headed Gull/Akyrau/773/2004 H13N6 GU982284
A/Great Black-headed Gull/Akyrau/744/2004 H13N6 GU982282
A/Black-headed Gull/Sweden/10/2005 H13N6 KR087578
A/Herring Gull/Finland/9875/2005 H13 KX455108
A/Duck/Hokkaido/W189/2006 H13N6 LC339627
A/Duck/Hokkaido/WZ68/2012 H13N2 AB812744
A/Duck/Interior Alaska/11P00703/2011 H13-mixed CY195637
A/Glaucous-winged Gull/Southcentral Alaska/11JR021820/2011 H13-mixed CY195628
A/Glaucous-winged Gull/SouthesasAlaska/JR082260/2009 H13N6 CY130340
A/Glaucous-winged Gull/Southcentral Alaska/9JR069R1/2009 H13N6 CY070850
A/Glaucous-winged Gull/Southcentral Alaska/9JR073R1/2009 H13N6 CY070858
A/Glaucous-winged Gull/Southcentral Alaska/9JR076R1/2009 H13N6 CY070874
A/Glaucous-winged Gull/Southcentral Alaska/9JR074R1/2009 H13N6 CY070866
A/Black-headed Gull/Netherlands/2/2007 H13N6 KR087579
A/Black-headed Gull/Netherlands/10/2007 H13N6 KR087582
A/Black-headed Gull/Netherlands/6/2007 H13N3 KR087581
A/Herring Gull/Finland/9330/2007 H13 KX455109
A/Herring Gull/Finland/9611/2007 H13 KX455110
A/Herring Gull/Norway/1023360/2006 H13N6 FM179758
A/Mongolian Gull/Mongolia/401/2007 H13N6 GQ907310
A/Mongolian Gull/Mongolia/405/2007 H13N6 GQ907318
A/Black-headed Gull/Georgia/7/2011 H13N6 KC541687
A/Yellow-legged Gull/Georgia/1/2010 H13N2 KC541676
A/Yellow-legged Gull/Republic of Georgia/1/2012 mixed CY185371
A/Yellow-legged Gull/Republic of Georgia/2/2012 H13N6 CY185601
A/Yellow-legged Gull/Republic of Georgia/5/2012 H13N6 CY185673
A/Black-headed Gull/Netherlands/27/2009 H13N2 KX978876
A/Black-headed Gull/Netherlands/34/2009 H13N2 MF682944
A/Black-headed Gull/Netherlands/8/2009 H13N2 MF147869
A/Black-headed Gull/Netherlands/18/2009 H13N2 KX978980
A/Black-headed Gull/Netherlands/7/2009 H13N2 KR087564
A/Common Gull/Norway/10/1313/2009 H13N2 HE802715
A/Black-headed Gull/Netherlands/12/2010 H13N2 KX978913
A/Black-headed Gull/Netherlands/13/2010 H13N2 KX979541
A/Black-headed Gull/Netherlands/39/2009 H13N6 KX979208
A/Black-headed Gull/Netherlands/17/2009 H13N3 KX978976
A/Black-headed Gull/Netherlands/31/2009 H13N2 KX979360
A/Yellow-legged Gull/Republic of Georgia/3/2012 H13N6 CY185609
A/Black-headed Gull/Georgia/5/2012 H13N6 MF682948
A/Yellow-legged Gull/Georgia/1/2011 H13N6 KC541688
A/Eurasian curlew/Liaoning/ZH-186/2014 H13N6 KR010435
A/Black-tailed Gull/Weihai/4/2016 H13N2 MH601562
A/Black-tailed Gull/Weihai/115/2016 H13N2 MF461180
A/Gull/Minnesota/945/1980 H13N6 CY014720
A/Gull/MD/181/78 H13N6 KF612933
A/Gull/MD/302/78 H13N6 KF612945
A/Gull/MD/490/79 H13N6 KF612947
A/Gull/MD/504/80 H13N6 KF612941
A/Gull/MA/18/80 H13N6 KF612943
A/Gull/MN/130/81 H13N6 KF612944
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A/Gull/MD/16/85 H13N2 KF612946
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A/Herring Gull/NJ/7321/88 H13N2 CY005932
A/Knot/DE/530/88 H13N6 KF612925
A/Crab eggs/DE/261/87 H13N2 KF612922
A/Laughing Gull/DE/157/89 H13N2 KF612923
A/Herring Gull/DE/237/88 H13N2 KF612924
A/Laughing Gull/Delaware/Bay/2838/1987 H13N2 CY101422
A/Laughing Gull/DE/246/93 H13N6 KF612931
A/Laughing Gull/DE/554/88 H13N3 KF612926
A/Ruddy Turnstone/DE/284/87 H13N2 KF612927
A/Herring Gull/DE/13/90 H13N2 KF612928
A/Ruddy Turnstone/Delaware/Bay/520/1988 H13N9 CY126288
A/Crab egg/DE/234/88 H13N6 KF612928
A/Herring Gull/NJ/163/90 H13-mixed KF612929
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A/Turkey/MN/1012/91 H13N2 KF612932
A/Herring Gull/DE/475/1988 H13N2 CY005914
A/Gull/MD/148/92 H13-mixed KF612935
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A/Red Knot/NJ/321/89 H13N4 KF612940
A/Laughing Gull/DE/242/87 H13N2 KF612939
A/Gull/NJ/34/92 H13N6 KF612936
A/Laughing Gull/DE/254/93 H13N1 KF612937
A/Ruddy Turnstone/DE/179/94 H13N3 KF612955
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A/Shorebird/DE/224/07 H13N6 KF612952
A/Sandpiper/DE/2516/87 H13N2 KF612951
A/Ring-billed Gull/Quebec/02622-1/2009 H13-mixed CY125317
A/Mallard Black hybrid/Newbrunswick/3736/2009 H13N6 CY128958
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A/Black-legged Kittiwake/Quebec/02838-1/2009 H13 CY125301
A/Gull/Massachusetts/12JR00601/2012 H13N6 CY195663
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A/Ruddy Turnstone/New Jersey/UGA/14-1436/2014 H13N6 MH502664
A/Laughing Gull/New Jersey/UGA17-2838/2017 H13N6 MH068335
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A/Georgia/NWRC/184017-60/2006 H13N2 CY122522
A/Shorebird/DelawareBay/424/2007 H13N9 CY127799
A/Florida/NWRC/183756-24/2006 H13N2 CY122451
A/NewYork/NWRC/182181-12/2006 H13N2 CY122459
A/Alabama/NWRC/183836-18/2006 H13N2 CY122492
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A/Glaucous-winged Gull/Alaska/667/2013 H13N2 KY131136
A/Black-headed Gull/Netherlands/19/2008 H13N8 MF146117
A/Black-headed Gull/Netherlands/3/2008 H13N8 MF146010
A/Black-headed Gull/Netherlands/48/2008 H13N8 MF682688
A/Black-headed Gull/Netherlands/18/2008 H13N8 MF147099
A/Black-headed Gull/Netherlands/21/2008 H13N8 KX978810
A/Black-headed Gull/Netherlands/22/2008 H13N8 MF145892
A/Black-headed Gull/Netherlands/10/2008 H13N8 MF682786
A/Black-headed Gull/Netherlands/35/2008 H13N8 MF682617
A/Black-headed Gull/Netherlands/38/2008 H13N8 MF146202
A/Black-headed Gull/Netherlands/25/2008 H13N8 KX979296
A/Black-headed Gull/Netherlands/49/2008 H13N8 MF147079
A/Black-headed Gull/Netherlands/51/2008 H13N8 MF147023
A/Black-headed Gull/Netherlands/86/2008 H13N8 MF146676
A/Black-headed Gull/Netherlands/52/2008 H13N8 MF145940
A/Black-headed Gull/Netherlands/94/2008 H13N8 KX978300
A/Black-headed Gull/Netherlands/102/2008 H13N8 KX978024
A/Black-headed Gull/Netherlands/96/2008 H13N8 KX979163
A/Black-headed Gull/Netherlands/24/2011 H13N8 MF575295
A/Black-headed Gull/Netherlands/23/2011 H13N8 MF575221
A/Black-headed Gull/Netherlands/25/2011 H13N8 MF147953
A/Black-headed Gull/Netherlands/2/2011 H13N8 KX979383
A/Black-headed Gull/Netherlands/10/2011 H13N8 MF147406
A/Black-headed Gull/Netherlands/12/2011 H13N8 MF146180
A/Black-headed Gull/Netherlands/15/2011 H13N8 KX979066
A/Black-headed Gull/Netherlands/18/2011 H13N8 KX977993
A/Black-headed Gull/Netherlands/21/2011 H13N8 KX978866
A/Black-headed Gull/Netherlands/4/2011 H13N8 KR087584
A/Black-headed Gull/Netherlands/5/2011 H13N8 KR087587
A/Black-headed Gull/Netherlands/26/2011 H13N8 KR087591
A/Black-headed Gull/Netherlands/3/2011 H13N8 KR087583
A/Black-headed Gull/Netherlands/20/2011 H13N8 KR087590
A/Black-headed Gull/Netherlands/35/2011 H13N8 KR087592
A/Black-headed Gull/Netherlands/8/2011 H13N3 KR087586
A/Black-headed Gull/Netherlands/38/2011 H13N8 KR087594
A/Black-headed Gull/Netherlands/17/2011 H13N8 KR087589
A/Black-headed Gull/Netherlands/16/2011 H13N8 KR087588
A/Black-headed Gull/Netherlands/36/2011 H13N8 KR087593
A/Black-headed Gull/Netherlands/5/2011 H13N8 KR087585
A/Slender billed gull/CH52/111/2008 H13N2 KU684463
A/Black-headed Gull/Netherlands/13/2014 H13N2 MF147040
A/Black-headed Gull/Netherlands/1/2014 H13N2 KX978817
A/Black-headed Gull/Netherlands/12/2014 H13N2 KX978367
A/Herring Gull/Mongolia/454/2008 H13N8 JF775470
A/Black-headed Gull/Netherlands/33/2009 H13N2 KX978020
A/Black-headed Gull/Netherlands/11/2009 H13N6 KX979019
A/Black-headed Gull/Netherlands/13/2009 H13N2 MF145916
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A/Black-headed Gull/Netherlands/5/2009 H13N2 KX979255
A/Black-headed Gull/Netherlands/6/2009 H13N2 MF147945
A/Black-headed Gull/Netherlands/1/2009 H13N2 KX979507
A/Black-headed Gull/Netherlands/37/2009 H13N2 KX978043
A/Black-headed Gull/Netherlands/20/2009 H13N2 KR087598
A/Black-headed Gull/Netherlands/38/2009 H13N2 MF148103
A/Black-headed Gull/Netherlands/83/2008 H13N8 KX979576
A/Black-headed Gull/Netherlands/88/2008 H13N8 MF147363
A/Black-headed Gull/Netherlands/85/2008 H13N8 MF682781
A/Black-headed Gull/Netherlands/87/2008 H13N8 MF145996
A/Black-headed Gull/Netherlands/67/2008 H13N8 MF146670
A/Black-headed Gull/Netherlands/24/2008 H13N8 MF147255
A/Black-headed Gull/Netherlands/64/2008 H13N8 MF147491
A/Black-headed Gull/Netherlands/60/2008 H13N8 MF145978
A/Black-headed Gull/Netherlands/44/2008 H13N8 MF146202
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A/Black-headed Gull/Netherlands/30/2008 H13N8 MF146174
A/Black-headed Gull/Netherlands/4/2008 H13N8 KR087601
A/Black-headed Gull/Netherlands/10/2013 H13N8 KX977721
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A/Black-headed Gull/Netherlands/122/2012 H13N8 MF146479
A/Black-headed Gull/Netherlands/22/2012 H13N8 MF145799
A/Black-headed Gull/Netherlands/10/2012 H13N8 MF147689
A/Black-headed Gull/Netherlands/18/2012 H13N8 MF147647
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A/Black-headed Gull/Netherlands/26/2012 H13N8 MF147342
A/Black-headed Gull/Netherlands/1/2012 H13N8 MF146992
A/Black-headed Gull/Netherlands/128/2012 H13N8 MF147553
A/Black-headed Gull/Netherlands/29/2012 H13N8 MF146506
A/Black-headed Gull/Netherlands/21/2012 H13N8 MF147878
A/Black-headed Gull/Netherlands/20/2012 H13N8 MF146063
A/Black-headed Gull/Netherlands/17/2012 H13N8 MF147189
A/Black-headed Gull/Netherlands/123/2012 H13N8 MF146534
A/Black-headed Gull/Netherlands/125/2012 H13N8 MF146435
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A/Black-headed Gull/Netherlands/79/2012 H13N8 MF147375
A/Black-headed Gull/Netherlands/80/2012 H13N8 MF147078
A/Black-headed Gull/Netherlands/117/2012 H13N8 KX978539
A/Black-headed Gull/Netherlands/75/2012 H13N8 MF147095
A/Black-headed Gull/Netherlands/115/2012 H13N6 KX978183
A/Black-headed Gull/Netherlands/90/2012 H13N8 MF147768
A/Black-headed Gull/Netherlands/88/2012 H13N8 KX978218
A/Black-headed Gull/Netherlands/100/2012 H13N8 KX978985
A/Black-headed Gull/Netherlands/86/2012 H13N8 MF147754
A/Black-headed Gull/Netherlands/83/2012 H13N8 KX977618
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A/Black-headed Gull/Netherlands/39/2012 H13N8 MF146672
A/Black-headed Gull/Netherlands/22/2012 H13N8 KX977772
A/Black-headed Gull/Netherlands/106/2012 H13N8 KX978718
A/Black-headed Gull/Netherlands/102/2012 H13N8 KX977943
A/Black-headed Gull/Netherlands/103/2012 H13N8 MF147266
A/Black-tailed Gull/Weihai/17/2016 H13N8 MF461188
A/Glaucous-winged Gull/Alaska/UGA15-6732/2015 H13-mixed KY131288
A/Glaucous-winged Gull/Southcentral Alaska/15MB02016/2015 H13N8 CY213695
A/Yellow-legged Gull/Georgia/2/2013 H13N8 CY185633
A/Yellow-legged Gull/Georgia/1/2013 H13N8 CY185625
A/Eurasian curlew/Liaoning/ZH-385/2014 H13N8 KR010443
A/Black-headed Gull/Netherlands/22/2016 H13N2 MF694026
A/Black-headed Gull/Netherlands/6/2016 H13N2 MF694199
A/Black-headed Gull/Netherlands/10/2010 H13N2 MF694241
A/Black-headed Gull/Netherlands/11/2016 H13N2 MF694057
A/Black-headed Gull/Netherlands/33/2014 H13N8 MF575196
A/Black-headed Gull/Netherlands/10/2014 H13N6 KX979051
A/Black-headed Gull/Netherlands/16/2014 H13N6 MF146531
A/Black-headed Gull/Netherlands/34/2014 H13N6 MF575309
A/Black-headed Gull/Netherlands/14/2014 H13N6 MF147981
A/Black-headed Gull/Netherlands/35/2014 H13N6 KX978735
A/Black-headed Gull/Netherlands/29/2014 H13N6 KX978504
A/Black-headed Gull/Netherlands/5/2014 H13N8 KX978389
A/Black-headed Gull/Netherlands/15/2014 H13N6 KX978686
A/Black-headed Gull/Netherlands/28/2014 H13N6 KX979165
A/Ring-billed Gull/Massachusetts/13DC30736-1/2013 H13N8 CY195801
A/Gull/New Jersey/UGA15-3767/2015 H13N3 MH501054
A/Franklins gull/Chile/C17421/2016 H13N9 MH498978
A/Franklins gull/Chile/C2300/4/2016 H13N9 MH499041
A/Elegant /Chile/C20085/2016 H13N9 MH499011
A/Franklins gull/Chile/C20069/2016 H13N9 MH498793
A/Franklins gull/Chile/C20118/2016 H13N9 MH499132
A/Franklins gull/Chile/C20110/2016 H13N9 MH499155
A/Franklins gull/Chile/C20149/2016 H13N9 MH499078
A/Black skimmer/Chile/C20124/2016 H13N9 MH498871
A/Black skimmer/C2008/4/2016 H13N9 MH498752
A/American oystercatcher/Chile/C20102/2016 H13N9 MH499091
A/Black skimmer/Chile/C20123/2016 H13N9 MH499019
A/Franklins gull/Chile/C20070/2016 H13N9 MH498930
A/Whimbrel/Chile/C20075/2016 H13N9 MH498968
A/Black skimmer/Chile/C20077/2016 H13N9 MH499127
A/Whimbrel/Chile/C20106/2016 H13N9 MH498956
A/Kelp gull/Chile/C20137/2016 H13N9 MH498851
A/Franklins gull/Chile/C20086/2016 H13N9 MH498907
A/White-backed stilt/Chile/C20062/2016 H13N9 MH498671
A/Black skimmer/Chile/C20142/2016 H13N9 MH499225
A/Black skimmer/Chile/C20100/2016 H13N9 MH499240
A/Franklins gull/Chile/C20073/2016 H13N9 MH499160
A/Blackish oystercatcher/Chile/C20060/2016 H13N9 MH498730
A/Whimbrel/Chile/C20144/2016 H13N9 MH499009
A/Franklins gull/Chile/C20061/2016 H13N9 MH498647
0.07

Figure S2


A/SilverGull/Tasmania/62/2006, H13N6 (CY094903)
A/Gull/MD/704/77_H13N6_(KF612959)
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A/Larus_ichthyaetus/Astrakhan/44/1988_H13N6_(EU564115)
A/Gull/ND/44030/92_H13N6_(KF612960)
A/Gull/Astrakhan/568/1990_H13N6_(EU835896)
A/Kelp_gull/Argentina/LDC4/2006_H13N9_(EU523136)
A/Duck/Siberia/272/1998_H13N6_(AB294988)
A/Great_black-backed_Gull/Sweden/1/2003_H13N6_(KR087577)
A/Gull/Astrakhan/34/2002_H13N6_(EU835897)
A/Great_Black-headed_Gull/Akyrai/773/2004_H13N6_(GU982284)
A/Herring_Gull/Finland/9875/2005_H13_(KX455108)
A/Black-headed_Gull/Sweden/102/2005_H13N6_(KR087578)
A/Duck/Hokkaido/W189/2006_H13N6_(LC339627)
A/Duck/Hokkaido/W268/2012_H13N2_(AB812744)
A/Duck/Interior_Alaska/13P002/03/2011_H13-mixed_(CY195637)
A/Glaucous-winged_Gull/Southcentral_Alaska/11JB02/16/2011_H13-mixed_(CY195628)
A/Glaucous-winged_Gull/Southeastern Alaska/9JR082/28/2009_H13N6_(CY130340)
A/Glaucous-winged_Gull/Southcentral Alaska/9JR073/81/2009_H13N6_(CY070858)
A/Glaucous-winged_Gull/Southcentral Alaska/9JR069/1R1/2009_H13N6_(CY070850)
A/Glaucous-winged_Gull/Southcentral Alaska/9JR079/81/2009_H13N6_(CY070874)
A/Glaucous-winged_Gull/Southcentral Alaska/9JR074/R1/2009_H13N6_(CY070866)
A/Great_Black-headed_Gull/Akyrai/744/2004_H13N6_(GU982262)
A/Black-headed_Gull/Netherlands/6/2007_H13N3_(KR087581)
A/Black-headed_Gull/Netherlands/2/2007_H13N6_(KR087579)
A/Black-headed_Gull/Netherlands/10/2007_H13N6_(KR087582)
A/Herring_Gull/Norway/1023362/2006_H13N6_(FM179758)
A/Herring_Gull/Finland/953/2007_H13_(KX455109)
A/Herring_Gull/Finland/9611/2007_H13_(KX456110)
A/Mongolian_Gull/Mongolia/405/2007_H13N6_(GQ907318)
A/Mongolian_Gull/Mongolia/401/2007_H13N6_(GQ907310)
A/Black-headed_Gull/Georgia/7/2011_H13N6_(KC541687)
A/Yellow-legged_Gull/Georgia/1/2010_H13N2_(KC541676)
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A/Yellow-legged_Gull/Republic_of_Georgia/1/2012_mixed_(CY185371)
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A/Black-headed_Gull/Netherlands/8/2009_H13N2_(MF147869)
A/Black-headed_Gull/Netherlands/7/2009_H13N2_(KR087564)
A/Black-headed_Gull/Netherlands/18/2009_H13N2_(KX097880)
A/Common_Gull/Norway/101313/2009_H13N2_(HE802715)
A/Black-headed_Gull/Netherlands/13/2010_H13N2_(KX097541)
A/Black-headed_Gull/Netherlands/12/2010_H13N2_(KX097813)
A/Black-headed_Gull/Netherlands/17/2009_H13N2_(KX097876)
A/Black-headed_Gull/Netherlands/39/2009_H13N6_(KX097208)
A/Black-headed_Gull/Netherlands/31/2009_H13N2_(KX097938)
A/Yellow-legged_Gull/Georgia/1/2011_H13N6_(KC541688)
A/Black-headed_Gull/Georgia/5/2012_H13N6_(MF682648)
A/Yellow-legged_Gull/Republic_of_Georgia/3/2012_H13N6_(CY185609)
A/Eurasian_curlew/Liaoning/ZH-180/2014_H13N6_(KR010435)
A/Black-tailed_Gull/Weihai/42/2016_H13N2_(MH291562)
A/Black-tailed_Gull/Weihai/115/2016_H13N2_(MF461180)
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A/Gull/MD/5049/80_H13N6_(KF612941)
A/Gull/MD/3027/78_H13N9_(KF612949)
A/Gull/Minnesota/945/1980_H13N6_(CY014720)
A/Gull/MD/185/78_H13N6_(KF612933)
A/Gull/MA/18/80_H13N6_(KF612943)
A/Gull/MN/13025/1_H13N6_(KF612944)
A/Gull/ME/16/88_H13N2_(KF612946)
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A/Herring_Gull/Quebec/02622-12/2009_H13-mixed_(CY125309)
A/Herring_Gull/Quebec/02638-12/2009_H13_(CY125301)
A/Mallard_Black_hybrid/NewBrunswick/3736/2009_H13N6_(CY128958)
A/Gull/Delaware/A09-435/2009_H13-mixed_(CY145687)
A/Ruddy_Turnstone/Delaware/2/1988_H13N6_(CY126288)
A/Herring_Gull/Delaware/990/1988_H13N6_(CY014603)
A/Turkey/MN/101291_H13N2_(KF612932)
A/Herring_Gull/NJ/163/90_H13-mixed_(KF612929)
A/Herring_Gull/DE/471/88_H13N2_(KF612934)
A/Gull/NJ/48/92_H13-mixed_(KF612935)
A/Herring_Gull/DE/2591/87_H13N2_(KF612930)
A/Laughing_Gull/DE/254/93_H13N1_(KF612937)
A/Gull/NJ/3492_H13N6_(KF612936)
A/Laughing_Gull/DE/424/87_H13N2_(KF612939)
A/Red_Knot/NJ/321/89_H13N6_(KF612940)
A/Ruddy_Turnstone/DE/179/94_H13N3_(KF612955)
A/Ring_billed_Gull/Georgia/A09-2958/2009_H13N6_(CY144202)
A/Sandpiper/DE/2516/87_H13N2_(KF612951)
A/Shorebird/DE/224/97_H13N6_(KF612952)
A/Great_black-backed_Gull/Newfoundland/296/2008_H13N2_(GU724153)
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A/Ring billed_Gull/Quebec/02638-12/2009_H13_(CY125301)
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A/Laughing_Gull/New_Jersey/UGA17-2839/2017_H13N6_(MH968335)
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A/Shorebird/DE/242/2009_H13N6_(KF612949)
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A/Environment/NewYorkNWRC182181-12/2006_H13N2_(CY122459)
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A/Environment/Georgia/NWRC184017-60/2006_H13N2_(CY122502)
A/Environment/Florida/NWRC183795-24/2006_H13N2_(CY122491)
A/Environment/Alabama/NWRC183538-18/2006_H13N2_(CY122460)
A/Glaucous-winged_Gull/Southcentral_Alaska/15MB0163/2015_H13N6_(CY213636)
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A/Gull/Astrakhan/1818/1998_H13N6_(EU835900)
A/Larus_minutus/Astrakhan/3357/2002_H13N2_(EU564108)
A/Black-headed_Gull/Sweden/2/2003_H13N8_(KR087600)
A/Black-headed_Gull/Sweden/1/2003_H13N8_(KR087599)
A/Black-headed_Gull/Mongolia/1766/2006_H13N6_(GQ907302)
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A/Lesser_snow_Goose/Alaska/44198-115/2006_H13N9_(HM059997)
A/Glaucous_Gull/Alaska/44199-104/2006_H13N9_(HM059996)
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A/Black-headed_Gull/Netherlands/1/2010_H13N8_(KX977814)
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A/Black-headed_Gull/Netherlands/8/2010_H13N2_(KX979026)
A/Yellow-legged_Gull/Georgia/1/2011_H13N8_(MF146200)
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A/Blackish_oystercatcher/Chile/C200062/2016_H13N9_(MH498730)
A/Whimbrel/Chile/C20144/2016_H13N9_(MH499009)
A/Frankins_gull/Chile/C20001/2016_H13N9_(MH498647)
A/Frankins_gull/Chile/C20118/2016_H13N9_(MH499132)
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A/Whimbrel/Chile/C20106/2016_H13N9_(MH498856)
A/Frankins_gull/Chile/C20149/2016_H13N9_(MH499078)
A/Black_skimmer/Chile/C20124/2016_H13N9_(MH498871)
A/Black_skimmer/Chile/C20142/2016_H13N9_(MH499225)
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A/White-backed_stilt/Chile/C20090/2016_H13N9_(MH498871)
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A/Frankins_gull/Chile/C20110/2016_H13N9_(MH499155)
A/Whimbrel/Chile/C20075/2016_H13N9_(MH498668)
A/Black_skimmer/Chile/C200770/2016_H13N9_(MH499127)
A/Frankins_gull/Chile/C20070/2016_H13N9_(MH498930)
A/American_oystercatcher/Chile/C201020/2016_H13N9_(MH499091)
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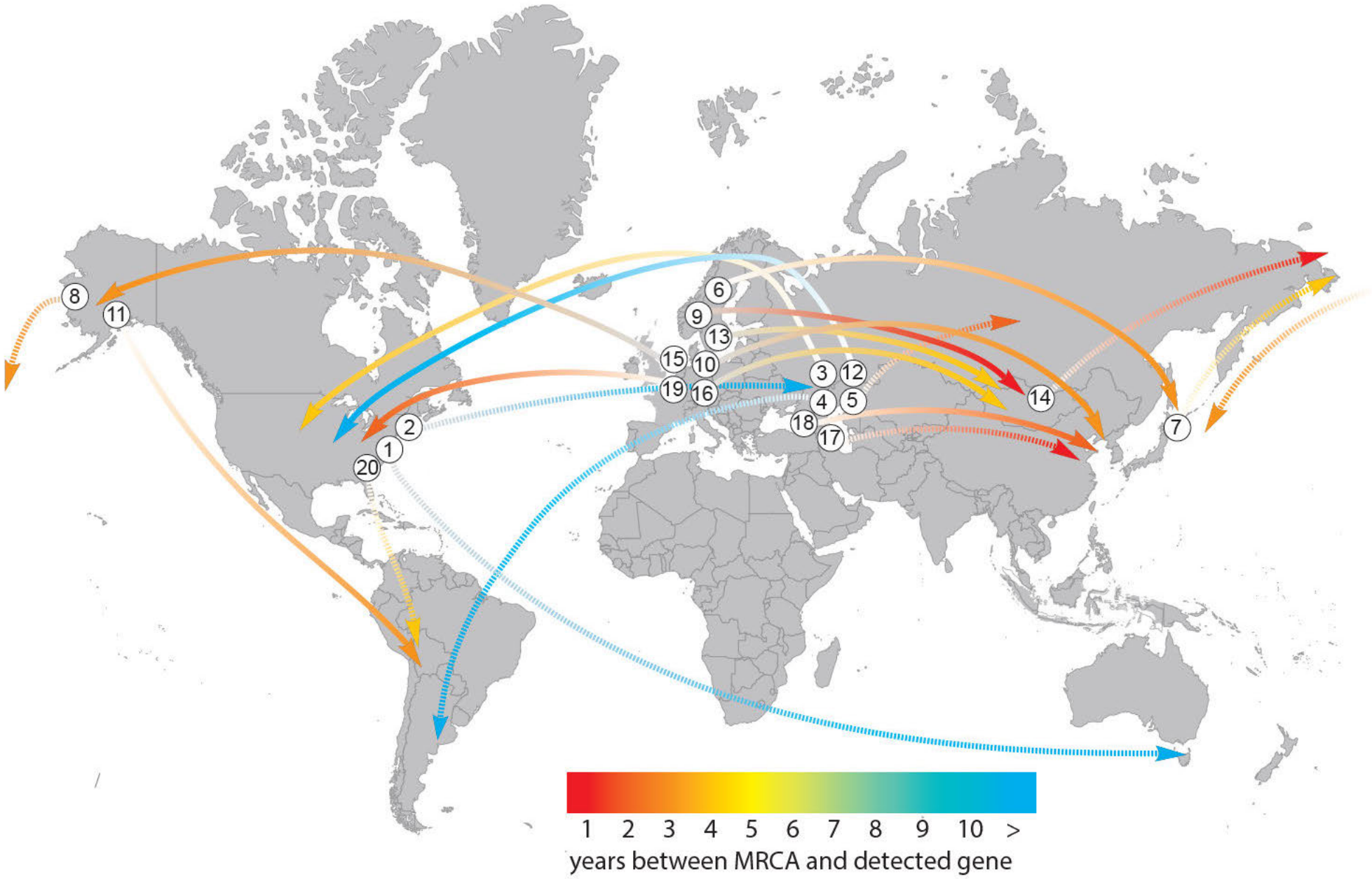
A/Black_skimmer/Chile/C20123/2016_H13N9_(MH499019)

Figure S3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years between MRCA and detected gene

Figure S4


A/Mallard/Gurjev/785/1983 H16N3 EU148600
A/Black-headed Gull/Sweden/4/1999 H16N3 AY684890
A/Black-headed Gull/Sweden/2/1999 H16N3 AY684888
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A/Black-headed Gull/Sweden/9476/2005 H16N3 KR087605
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A/Black-headed Gull/Netherlands/9/2007 H16N3 KR087613
A/Black-headed Gull/Netherlands/8/2007 H16N3 KR087612
A/Black-headed Gull/Netherlands/5/2007 H16N3 KR087611
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A/Black-headed Gull/Netherlands/114/2012 H16N3 KX977892
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A/Black-headed Gull/Netherlands/3/2016 H16N3 MF694124
A/Black-headed Gull/Netherlands/1/2016 H16N3 MF694134
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A/Black-headed Gull/Netherlands/29/2008 H16N3 MF146193
A/Black-headed Gull/Netherlands/28/2008 H16N3 MF146238
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A/Black-headed Gull/Netherlands/4/2015 H16N3 KX978525
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A/Environment/Alaska/NWRC184854-12/2006 H16N3 CY122507
A/Fulica atra/Volga/635/1986 H16N3 EU564109
A/Black-headed Gull/Turkmenistan/13/1976 H16N3 EU293864
A/Teal/Volga/671/1986 H16N3 EU148602
A/Slender-billed Gull/Astrakhan/28/1976 H16N3 EU293865
A/Black-headed Gull/Sweden/5/1999 H16N3 AY684891
A/Duck/Hokkaido/WZ82/2013 H16N3 LC339707
A/Little Tern/Sweden/55316/2006 H16N3 KR087616
A/wildbird/Sweden/1/2005 H16N3 KR087602
A/wildbird/Sweden/2/2005 H16N3 KR087603
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A/Herring Gull/NewJersey/780/1986 H16N3 CY136590
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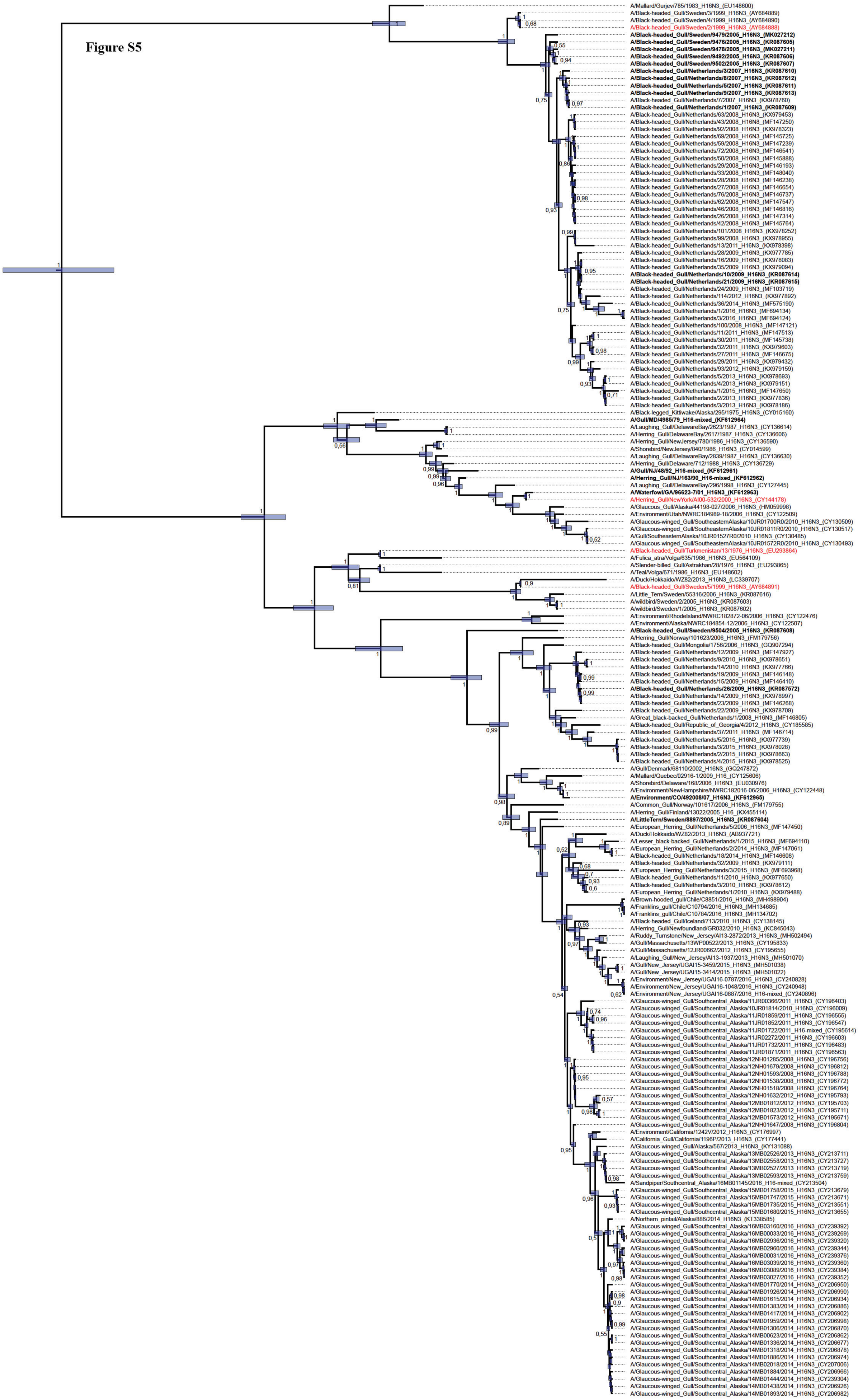
Figure S5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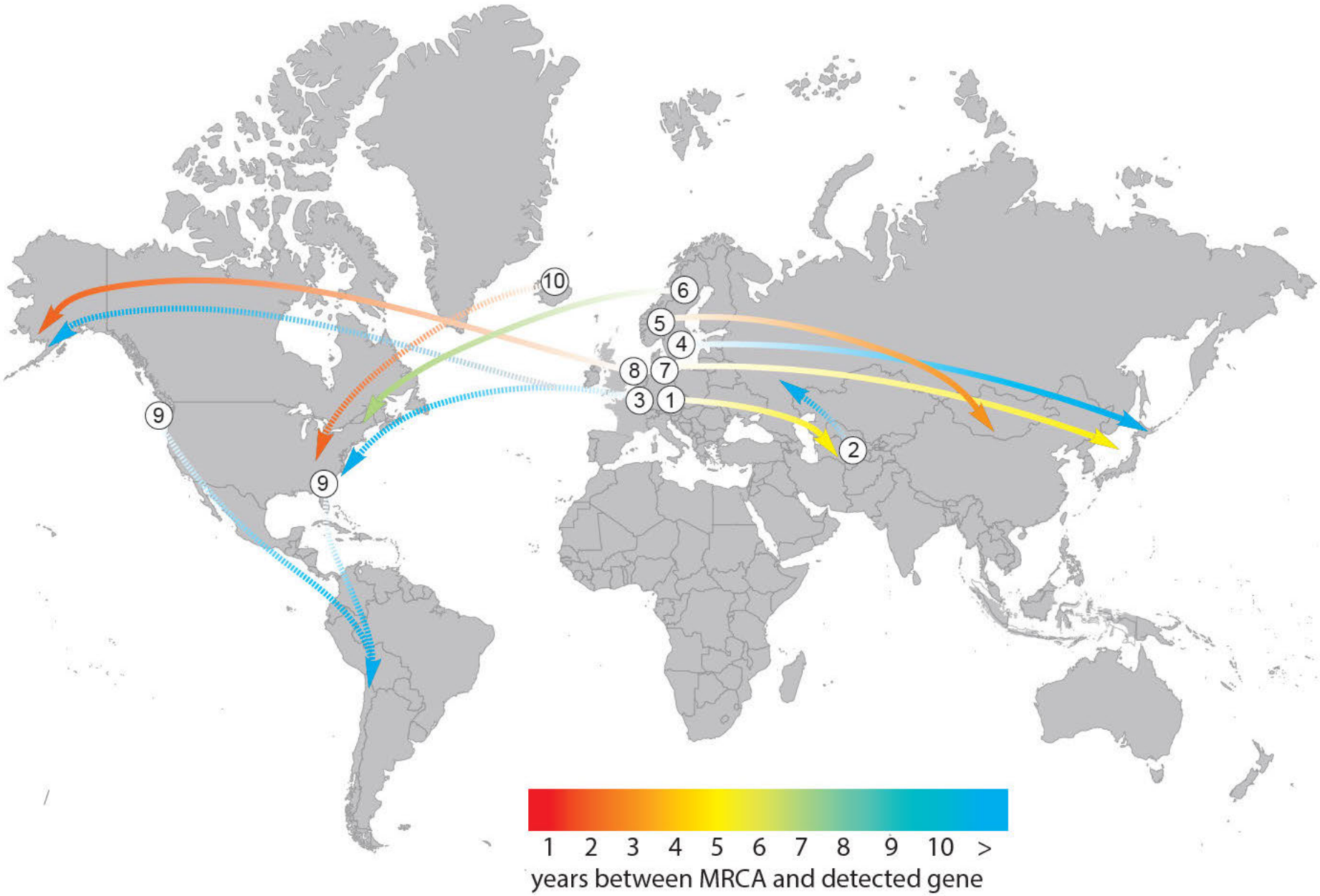
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01615/2014_H16N3_(CY206934)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01383/2014_H16N3_(CY206886)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01417/2014_H16N3_(CY206902)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01959/2014_H16N3_(CY206998)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01306/2014_H16N3_(CY206870)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB00623/2014_H16N3_(CY206862)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01336/2014_H16N3_(CY206677)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01318/2014_H16N3_(CY206878)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01886/2014_H16N3_(CY206974)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB02018/2014_H16N3_(CY207006)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01884/2014_H16N3_(CY206966)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01444/2014_H16N3_(CY239304)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01438/2014_H16N3_(CY206926)
A/Glaucous-winged_Gull/Southcentral_Alaska/14MB01893/2014_H16N3_(CY206982)
10

Figure S6


10
6
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1 2 3 4 5 6 7 8 9 10 >
years between MRCA and detected gene

Tabel S1

Host		Region	No. Subtype (%)																Total
Common name	**Latin name**		**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**	**H14**	**H15**	**H16**	
Armenian gull	*Larus armenicus*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	2 (100)	-	-	-	2 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Black-headed gull	*Chroicocephalus ridibundus*	Eurasia	1 (0)	3 (1)	1 (0)	1 (0)	8 (2)	1 (0)	-	-	5 (1)	1 (0)	3 (1)	-	334 (70)	-	-	119 (25)	477 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Black-tailed gull	*Larus crassirostris*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	4 (100)	-	-	-	4 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown-Headed gull	*Chroicocephalus brunnicephalus*	Eurasia	-	-	-	-	11 (100)	-	-	-	-	-	-	-	-	-	-	-	11 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown-hooded gull	*Chroicocephalus maculipennis*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	-	-	-	-	-	-	-	-	1 (20)	-	-	-	3 (60)	-	-	1 (20)	5 (100)
California gull	*Larus californicus*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	-	-	-	-	-	-	-	-	-	1 (50)	-	-	-	-	-	1 (50)	2 (100)
Dolphin gull	*Leucophaeus scoresbii*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (100)	1 (100)
Franklin's gull	*Leucophaeus pipixcan*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	-	-	-	-	-	1 (7)	-	-	-	-	-	-	11 (79)	-	-	2 (14)	14 (100)
Glaucous gull	*Larus hyperboreus*	Eurasia	-	2 (50)	-	-	-	-	-	-	-	2 (50)	-	-	-	-	-	-	4 (100)
		America	-	-	1 (13)	-	1 (13)	1 (13)	-	-	-	-	-	-	4 (50)	-	-	1 (13)	8 (100)
Glaucous-winged gull	*Larus glaucescens*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	1 (1)	-	4 (3)	3 (2)	4 (3)	-	-	-	-	-	2 (1)	-	41 (26)	-	-	101 (65)	156 (100)
Great black-backed gull	*Larus marinus*	Eurasia	-	2 (33)	-	1 (17)	1 (17)	-	-	-	-	-	-	-	1 (17)	-	-	1 (17)	6 (100)
		America	1 (20)	-	-	-	-	-	-	-	1 (20)	-	-	-	2 (40)	-	-	1 (20)	5 (100)
Great black-headed gull (Pallas's gull)	*Ichthyaetus ichthyaetus*	Eurasia	-	-	-	-	12 (55)	-	-	-	-	-	-	-	10 (45)	-	-	-	22 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Herring gull	*Larus argentatus*	America	1 (2)	7 (13)	1 (2)	1 (2)	3 (6)	3 (6)	-	-	-	3 (6)	4 (8)	-	22 (42)	-	-	8 (15)	53 (100)
		Eurasia	-	5 (12)	1 (2)	1 (2)	6 (14)	1 (2)	-	-	-	1 (2)	2 (5)	-	14 (33)	1 (2)	-	11 (26)	43 (100)
Iceland gull	*Larus glaucoides*	Eurasia	-	1 (33)	-	-	-	-	-	-	-	2 (67)	-	-	-	-	-	-	3 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kelp gull	*Larus dominicanus*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	-	-	1 (4)	-	2 (8)	3 (12)	-	-	-	-	-	-	13 (52)	-	-	6 (24)	25 (100)
Laughing gull	*Leucophaeus atricilla*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	2 (2)	7 (7)	8 (8)	1 (1)	3 (3)	16 (16)	9 (9)	-	7 (7)	4 (4)	7 (7)	2 (2)	27 (27)	-	-	8 (8)	101 (100)
Little gull	*Hydrocoloeus minutus*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	1 (100)	-	-	-	1 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mediterranean gull	*Ichthyaetus melanocephalus*	Eurasia	-	-	-	-	-	-	-	-	3 (75)	-	-	-	1 (25)	-	-	-	4 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mew Gull	*Larus canus*	Eurasia	-	-	-	-	1 (10)	1 (10)	-	-	-	-	-	-	4 (40)	-	-	4 (40)	10 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (100)	1 (100)
Ring-billed gull	*Larus delawarensis*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	5 (7)	-	-	-	-	3 (4)	-	-	-	-	2 (3)	-	55 (82)	-	-	2 (3)	67 (100)
Sabine'S gull	*Xema sabini*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		America	-	-	-	-	1 (100)	-	-	-	-	-	-	-	-	-	-	-	1 (100)
Slaty-backed gull	*Larus schistisagus*	Eurasia	-	-	-	2 (50)	2 (50)	-	-	-	-	-	-	-	-	-	-	-	4 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	1 (100)	-	-	-	-	1 (100)
Slender-Billed gull	*Chroicocephalus genei*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	1 (50)	-	-	1 (50)	2 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yellow-legged gull	*Larus michahellis*	Eurasia	-	-	-	-	-	-	-	-	-	-	-	-	13 (100)	-	-	-	13 (100)
		America	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unknown gull species	Unknown gull species	Eurasia	-	-	2 (7)	2 (7)	3 (10)	2 (7)	3 (10)	-	-	-	2 (7)	-	13 (43)	-	-	3 (10)	30 (100)
		America	5 (6)	5 (6)	1 (1)	13 (16)	3 (4)	3 (4)	2 (3)	-	1 (1)	6 (8)	4 (5)	-	30 (38)	-	-	7 (9)	80 (100)
Total			16 (1)	32 (3)	20 (2)	25 (2)	61 (5)	35 (3)	14 (1)	-	18 (2)	20 (2)	26 (2)	-	606 (52)	1 (0)	-	279 (24)	1156 (100)

Table S2

Virus	Subtype	Accession number
A/Gull/MD/704/77	**H13N6**	**KF612959**
A/Gull/MD/1815/78	**H13N6**	**KF612933**
A/Gull/MD/3027/78	**H13N9**	**KF612945**
A/Great Black-headed Gull/Astrakhan/1420/1979	H13N2	EU293858
A/Great Black-headed Gull/Astrakhan/1421/1979	H13N2	EU293859
A/Gull/Astrakhan/1314/1979	H13N2	EU835898
A/Gull/MD/4909/79	**H13N6**	**KF612947**
A/Gull/MD/4985/79	**H13-mixed**	**KF612942**
A/Gull/MA/18/80	**H13N6**	**KF612943**
A/Gull/MD/5049/80	**H13N6**	**KF612941**
A/Gull/Minnesota/945/1980	H13N6	CY014720
A/Gull/MN/1352/81	**H13N6**	**KF612944**
A/Great Black-headed Gull/Astrakhan/591/1982	H13N2	EU293860
A/Black-headed Gull/Astrakhan/65/1983	H13N6	EU580577
A/Great Black-headed Gull/Gurjev/76/1983	H13N2	EU293861
A/Larus ichthyaetus/Astrakhan/75/1983	H13N2	EU564107
A/Black-headed Gull/Astrakhan/227/1984	H13N6	M26089
A/Gull/Astrakhan/226/1984	H13N6	EU835895
A/Pilot Whale/Maine/328HN/1984	H13N2	M26091
A/Gull/ME/16/85	**H13N2**	**KF612946**
A/Herring Gull/Astrakhan/458/1985	H13N6	EU293862
A/Herring Gull/Astrakhan/479/1985	H13N6	EU293863
A/Gull/Astrakhan/176/1986	H13N2	EU835899
A/Herring Gull/DE/471/86	**H13N7**	**KF612934**
A/Herring Gull/DE/475/1986	H13N2	CY005914
A/Herring Gull/NJ/782/1986	H13N2	CY005932
A/Laughing Gull/DE/1370/86	**H13N2**	**KF612923**
A/Crab egg/DE/2618/87	**H13N2**	**KF612922**
A/Herring Gull/DE/2591/87	**H13N2**	**KF612930**
A/Laughing Gull/DE/2424/87	**H13N2**	**KF612939**
A/Laughing Gull/DelawareBay/2838/1987	H13N2	CY101422
A/Ruddy Turnstone/DE/2584/87	**H13N2**	**KF612927**
A/Sandpiper/DE/2516/87	**H13N2**	**KF612951**
A/Crab egg/DE/2347/88	**H13N6**	**KF612928**
A/Herring Gull/DE/2337/88	**H13N2**	**KF612924**
A/Herring Gull/Delaware/660/1988	H13N6	CY014603
A/Knot/DE/530/88	**H13N6**	**KF612925**
A/Larus ichthyaetus/Astrakhan/10/1988	H13N6	EU564106
A/Larus ichthyaetus/Astrakhan/44/1988	H13N6	EU564115
A/Laughing Gull/DE/554/88	**H13N3**	**KF612926**
A/RuddyTurnstone/DelawareBay/520/1988	H13N9	CY126288
A/Red Knot/NJ/321/89	**H13N4**	**KF612940**
A/Gull/Astrakhan/998/1990	H13N6	EU835896
A/Herring Gull/DE/13/90	**H13N2**	**KF612938**
A/Herring Gull/NJ/163/90	**H13-mixed**	**KF612929**
A/Turkey/MN/1012/91	**H13N2**	**KF612932**
A/Gull/ND/44036/92	**H13N6**	**KF612960**
A/Gull/NJ/34/92	**H13N6**	**KF612936**
A/Gull/NJ/48/92	**H13-mixed**	**KF612935**
A/Laughing Gull/DE/246/93	**H13N6**	**KF612931**
A/Laughing Gull/DE/254/93	**H13N1**	**KF612937**
A/Ruddy Turnstone/DE/179/94	**H13N3**	**KF612955**
A/Shorebird/DE/224/97	**H13N6**	**KF612952**
A/Duck/Siberia/272/1998	H13N6	AB284988
A/Gull/Astrakhan/1818/1998	H13N6	EU835900
A/Gull/Astrakhan/1846/1998	H13N6	EU580576
A/Black-headed Gull/Sweden/1/1999	H13N6	AY684887
A/Black-headed Gull/Netherlands/1/2000	H13N8	MF146968
A/Ring-billed Gull/Georgia/AI00-2658/2000	H13N6	CY144202

Virus	Subtype	Accession number
A/Shorebird/DE/188/2000	**H13N6**	**KF612948**
A/Gull/Astrakhan/3483/2002	H13N6	EU835897
A/Larus minutus/Astrakhan/3357/2002	H13N2	EU564108
A/Black-headed Gull/Sweden/1/2003	**H13N8**	**KR087599**
A/Black-headed Gull/Sweden/2/2003	**H13N8**	**KR087600**
A/Great black-backed Gull/**Sweden/1/2003**	**H13N6**	**KR087577**
A/Great Black-headed Gull/Atyrau/743/2004	H13N6	GU982281
A/Great Black-headed Gull/Atyrau/744/2004	H13N6	GU982282
A/Great Black-headed Gull/Atyrau/767/2004	H13N6	GU982283
A/Great Black-headed Gull/Atyrau/773/2004	H13N6	GU982284
A/Shorebird/DE/68/2004	H13N9	CY005931
A/Black-headed Gull/Sweden/1/2005	H13N8	CY077000
A/Black-headed Gull/Sweden/10/2005	**H13N6**	**KR087578**
A/Herring Gull/Finland/9875/2005	H13	KX455108
A/Black-headed Gull/Mongolia/1766/2006	H13N6	GQ907302
A/Black-headed Gull/Sweden/1/2006	**H13N8**	**KR087597**
A/Duck/Hokkaido/W189/2006	H13N6	LC339627
A/Environment/Alabama/NWRC183838-18/2006	H13N2	CY122492
A/Environment/Florida/NWRC183796-24/2006	H13N2	CY122491
A/Environment/Georgia/NWRC183417-30/2006	H13N2	CY122488
A/Environment/Georgia/NWRC184017-60/2006	H13N2	CY122502
A/Environment/NewYork/NWRC182181-12/2006	H13N2	CY122459
A/Environment/Ohio/NWRC182318-30/2006	H13N2	CY122462
A/Glaucous Gull/Alaska/44199-006/2006	H13N9	HM059994
A/Glaucous Gull/Alaska/44199-097/2006	H13N3	HM059995
A/Glaucous Gull/Alaska/44199-104/2006	H13N9	HM059996
A/Herring Gull/Massachusetts/A00080255/2006	H13N2	CY239408
A/Herring Gull/Massachusetts/A00080257/2006	H13N2	CY239280
A/Herring Gull/Norway/102336/2006	H13N6	FM179758
A/Kelp Gull/Argentina/LDC4/2006	H13N9	EU523136
A/Lesser snow Goose/Alaska/44199-115/2006	H13N9	HM059997
A/Shorebird/Delaware/221/2006	H13N9	CY043888
A/Shorebird/Delaware/224/2006	H13N9	CY043896
A/SilverGull/Tasmania/62/2006	H13N6	CY094903
A/American White Pelican/Minnesota/AI-07-1819/2007	H13N9	CY054300
A/Black-headed Gull/Netherlands/10/2007	**H13N6**	**KR087582**
A/Black-headed Gull/Netherlands/2/2007	**H13N6**	**KR087579**
A/Black-headed Gull/Netherlands/4/2007	**H13N6**	**KR087580**
A/Black-headed Gull/Netherlands/6/2007	**H13N3**	**KR087581**
A/Herring Gull/CT/1783-10/07	**H13N3**	**KF612954**
A/Herring Gull/Finland/9330/2007	H13	KX455109
A/Herring Gull/Finland/9611/2007	H13	KX455110
A/Mongolian Gull/Mongolia/401/2007	H13N6	GQ907310
A/Mongolian Gull/Mongolia/405/2007	H13N6	GQ907318
A/Shorebird/DelawareBay/424/2007	H13N9	CY127799
A/American White Pelican/Minnesota/Sg-0611/2008	H13N9	CY054302
A/Black-headed Gull/Netherlands/10/2008	H13N8	MF682786
A/Black-headed Gull/Netherlands/102/2008	H13N8	KX978024
A/Black-headed Gull/Netherlands/11/2008	H13N8	MF147313
A/Black-headed Gull/Netherlands/12/2008	H13N8	MF146078
A/Black-headed Gull/Netherlands/13/2008	H13N8	MF148122
A/Black-headed Gull/Netherlands/14/2008	H13N8	MF145859
A/Black-headed Gull/Netherlands/15/2008	H13N8	MF146408
A/Black-headed Gull/Netherlands/16/2008	H13N8	MF146171
A/Black-headed Gull/Netherlands/17/2008	H13N8	MF146229
A/Black-headed Gull/Netherlands/18/2008	H13N8	MF147099
A/Black-headed Gull/Netherlands/19/2008	H13N8	MF146117
A/Black-headed Gull/Netherlands/2/2008	H13N8	MF146364
A/Black-headed Gull/Netherlands/20/2008	H13N8	KX977714

Virus	Subtype	Accession number
A/Black-headed Gull/Netherlands/21/2008	H13N8	KX978810
A/Black-headed Gull/Netherlands/22/2008	H13N8	MF145892
A/Black-headed Gull/Netherlands/23/2008	H13N8	KX978852
A/Black-headed Gull/Netherlands/24/2008	H13N8	MF147255
A/Black-headed Gull/Netherlands/25/2008	H13N8	KX979286
A/Black-headed Gull/Netherlands/3/2008	H13N8	MF146870
A/Black-headed Gull/Netherlands/30/2008	H13N8	MF146174
A/Black-headed Gull/Netherlands/35/2008	H13N8	MF682817
A/Black-headed Gull/Netherlands/37/2008	H13N8	MF146360
A/Black-headed Gull/Netherlands/38/2008	H13N8	MF146262
A/Black-headed Gull/Netherlands/39/2008	H13N8	MF148072
A/Black-headed Gull/Netherlands/4/2008	**H13N8**	**KR087601**
A/Black-headed Gull/Netherlands/44/2008	H13N8	MF146202
A/Black-headed Gull/Netherlands/48/2008	H13N8	MF682688
A/Black-headed Gull/Netherlands/49/2008	H13N8	MF147079
A/Black-headed Gull/Netherlands/5/2008	H13N8	MF145989
A/Black-headed Gull/Netherlands/51/2008	H13N8	MF147023
A/Black-headed Gull/Netherlands/52/2008	H13N8	MF145940
A/Black-headed Gull/Netherlands/55/2008	H13N8	KX979227
A/Black-headed Gull/Netherlands/6/2008	H13N8	MF146566
A/Black-headed Gull/Netherlands/64/2008	H13N8	MF147491
A/Black-headed Gull/Netherlands/65/2008	H13N8	MF682781
A/Black-headed Gull/Netherlands/66/2008	H13N8	MF146391
A/Black-headed Gull/Netherlands/67/2008	H13N8	MF146670
A/Black-headed Gull/Netherlands/68/2008	H13N8	MF145978
A/Black-headed Gull/Netherlands/7/2008	H13N8	MF147648
A/Black-headed Gull/Netherlands/70/2008	H13N8	MF146424
A/Black-headed Gull/Netherlands/74/2008	H13N8	MF146501
A/Black-headed Gull/Netherlands/8/2008	H13N8	MF575016
A/Black-headed Gull/Netherlands/83/2008	H13N8	KX979576
A/Black-headed Gull/Netherlands/86/2008	H13N8	MF146676
A/Black-headed Gull/Netherlands/87/2008	H13N8	MF145996
A/Black-headed Gull/Netherlands/88/2008	H13N8	MF147363
A/Black-headed Gull/Netherlands/9/2008	H13N8	KX978340
A/Black-headed Gull/Netherlands/93/2008	H13N8	MF145945
A/Black-headed Gull/Netherlands/94/2008	H13N8	KX978300
A/Black-headed Gull/Netherlands/95/2008	H13N8	MF148068
A/Black-headed Gull/Netherlands/96/2008	H13N8	KX979163
A/Black-headed Gull/Netherlands/97/2008	H13N8	MF147665
A/Great black-backed Gull/Newfoundland/296/2008	H13N2	GU724153
A/Herring Gull/Mongolia/454/2008	H13N8	JF775470
A/Laughing Gull/AI08-0714/NJ/08	**H13N9**	**KF612956**
A/Laughing Gull/AI08-1388/NJ/08	**H13N9**	**KF612957**
A/Laughing Gull/AI08-1460/NJ/08	**H13N9**	**KF612958**
A/Black-headed Gull/Netherlands/1/2009	H13N2	KX979507
A/Black-headed Gull/Netherlands/11/2009	H13N6	KX979019
A/Black-headed Gull/Netherlands/13/2009	H13N2	MF145916
A/Black-headed Gull/Netherlands/17/2009	H13N3	KX978076
A/Black-headed Gull/Netherlands/18/2009	H13N2	KX978980
A/Black-headed Gull/Netherlands/2/2009	H13N2	MF147797
A/Black-headed Gull/Netherlands/20/2009	**H13N2**	**KR087598**
A/Black-headed Gull/Netherlands/27/2009	H13N2	KX978876
A/Black-headed Gull/Netherlands/29/2009	H13N6	KX979544
A/Black-headed Gull/Netherlands/3/2009	H13N2	MF146414
A/Black-headed Gull/Netherlands/31/2009	H13N2	KX979380
A/Black-headed Gull/Netherlands/33/2009	H13N2	KX978020
A/Black-headed Gull/Netherlands/34/2009	H13N2	MF682844
A/Black-headed Gull/Netherlands/36/2009	H13N2	MF147594
A/Black-headed Gull/Netherlands/37/2009	H13N2	KX978043

Virus	Subtype	Accession number
A/Black-headed Gull/Netherlands/38/2009	H13N2	MF148103
A/Black-headed Gull/Netherlands/39/2009	H13N6	KX979208
A/Black-headed Gull/Netherlands/4/2009	H13N2	MF959989
A/Black-headed Gull/Netherlands/5/2009	H13N2	MF146421
A/Black-headed Gull/Netherlands/6/2009	H13N2	MF147945
A/Black-headed Gull/Netherlands/7/2009	**H13N2**	**KR087564**
A/Black-headed Gull/Netherlands/8/2009	H13N2	MF147869
A/Black-headed Gull/Netherlands/9/2009	H13N2	KX978293
A/Black-legged Kittiwake/Quebec/02838-1/2009	H13	CY125301
A/Common Gull/Norway/101313/2009	H13N2	HE802715
A/Glaucous-winged Gull/SouthcentralAlaska/9JR0691R1/2009	H13N6	CY070850
A/Glaucous-winged Gull/SouthcentralAlaska/9JR0738R1/2009	H13N6	CY070858
A/Glaucous-winged Gull/SouthcentralAlaska/9JR0747R1/2009	H13N6	CY070866
A/Glaucous-winged Gull/SouthcentralAlaska/9JR0769R1/2009	H13N6	CY070874
A/Glaucous-winged Gull/SouthcentralAlaska/9JR0781R1/2009	H13N6	CY070882
A/Glaucous-winged Gull/SoutheasternAlaska/9JR0822R0/2009	H13N6	CY130340
A/Gull/Delaware/AI09-435/2009	H13-mixed	CY145987
A/Hooded Merganser/NewBrunswick/3750/2009	H13	CY125309
A/Mallard Black Duck hybrid/NewBrunswick/3736/2009	H13N6	CY128958
A/Ring-billed Gull/Quebec/02622-1/2009	H13-mixed	CY125317
A/Ruddy Turnstone/AI09-294/NJ/09	**H13N6**	**KF612950**
A/Shorebird/DE/204/2009	**H13N6**	**KF612949**
A/Slender-billed gull/CHBZ/11/2009	H13N2	KU684463
A/Black-headed Gull/Netherlands/1/2010	H13N6	KX977814
A/Black-headed Gull/Netherlands/10/2010	H13N8	KX978155
A/Black-headed Gull/Netherlands/12/2010	H13N2	KX978913
A/Black-headed Gull/Netherlands/13/2010	H13N2	KX979541
A/Black-headed Gull/Netherlands/2/2010	H13N8	MF146087
A/Black-headed Gull/Netherlands/4/2010	H13N8	KX977869
A/Black-headed Gull/Netherlands/5/2010	H13N8	MF145955
A/Black-headed Gull/Netherlands/6/2010	H13N8	KX979279
A/Black-headed Gull/Netherlands/7/2010	H13N8	MF682666
A/Black-headed Gull/Netherlands/8/2010	H13N2	KX979026
A/Mallard/Korea/SH38-45/2010	H13	JX030406
A/Ring-billed Gull/AI10-1708/MN/10	**H13N6**	**KF612953**
A/Yellow-legged Gull/Georgia/1/2010	H13N2	KC541676
A/Black-headed Gull/Georgia/1/2011	H13N8	KC541677
A/Black-headed Gull/Georgia/3/2011	H13N8	KC541680
A/Black-headed Gull/Georgia/6/2011	H13N8	KC541682
A/Black-headed Gull/Georgia/7/2011	H13N6	KC541687
A/Black-headed Gull/Netherlands/10/2011	H13N8	MF147406
A/Black-headed Gull/Netherlands/12/2011	H13N8	MF146180
A/Black-headed Gull/Netherlands/14/2011	H13N8	MF147288
A/Black-headed Gull/Netherlands/15/2011	H13N8	KX979066
A/Black-headed Gull/Netherlands/16/2011	**H13N8**	**KR087588**
A/Black-headed Gull/Netherlands/17/2011	**H13N8**	**KR087589**
A/Black-headed Gull/Netherlands/18/2011	H13N8	KX977693
A/Black-headed Gull/Netherlands/19/2011	H13N8	MF145917
A/Black-headed Gull/Netherlands/2/2011	H13N8	KX979383
A/Black-headed Gull/Netherlands/20/2011	**H13N8**	**KR087590**
A/Black-headed Gull/Netherlands/21/2011	H13N8	KX978666
A/Black-headed Gull/Netherlands/23/2011	H13N8	MF575221
A/Black-headed Gull/Netherlands/24/2011	H13N8	MF575295
A/Black-headed Gull/Netherlands/25/2011	H13N8	MF147953
A/Black-headed Gull/Netherlands/26/2011	**H13N8**	**KR087591**
A/Black-headed Gull/Netherlands/3/2011	**H13N8**	**KR087583**
A/Black-headed Gull/Netherlands/35/2011	**H13N8**	**KR087592**
A/Black-headed Gull/Netherlands/36/2011	**H13N8**	**KR087593**
A/Black-headed Gull/Netherlands/38/2011	**H13N8**	**KR087594**

Virus	Subtype	Accession number
A/Black-headed Gull/Netherlands/39/2011	**H13N8**	**KR087595**
A/Black-headed Gull/Netherlands/4/2011	**H13N8**	**KR087584**
A/Black-headed Gull/Netherlands/5/2011	**H13N8**	**KR087585**
A/Black-headed Gull/Netherlands/8/2011	**H13N3**	**KR087586**
A/Black-headed Gull/Netherlands/9/2011	**H13N8**	**KR087587**
A/Duck/Interior Alaska/11PG00703/2011	H13-mixed	CY195637
A/Glaucous-winged Gull/Southcentral Alaska/11JR02182/2011	H13-mixed	CY195628
A/Glaucous-winged Gull/Southcentral Alaska/11JR02474/2011	H13N6	CY196611
A/Yellow-legged Gull/Georgia/1/2011	H13N6	KC541688
A/Yellow-legged Gull/Georgia/1/2011	H13N8	MF146200
A/Black-headed Gull/Georgia/5/2012	H13N6	MF682848
A/Black-headed Gull/Netherlands/1/2012	H13N6	MF146992
A/Black-headed Gull/Netherlands/10/2012	H13N6	MF147689
A/Black-headed Gull/Netherlands/100/2012	H13N6	KX978985
A/Black-headed Gull/Netherlands/101/2012	H13N6	MF145970
A/Black-headed Gull/Netherlands/102/2012	H13N6	KX977943
A/Black-headed Gull/Netherlands/103/2012	H13N6	MF147266
A/Black-headed Gull/Netherlands/104/2012	H13N6	KX979504
A/Black-headed Gull/Netherlands/105/2012	H13N6	KX979468
A/Black-headed Gull/Netherlands/106/2012	H13N6	KX978718
A/Black-headed Gull/Netherlands/108/2012	H13N6	MF146166
A/Black-headed Gull/Netherlands/109/2012	H13N6	KX978793
A/Black-headed Gull/Netherlands/11/2012	H13N6	MF148049
A/Black-headed Gull/Netherlands/110/2012	H13N6	KX978037
A/Black-headed Gull/Netherlands/111/2012	H13N6	MF145899
A/Black-headed Gull/Netherlands/112/2012	H13N6	KX978433
A/Black-headed Gull/Netherlands/113/2012	H13N6	KX979591
A/Black-headed Gull/Netherlands/115/2012	H13N6	KX978183
A/Black-headed Gull/Netherlands/117/2012	H13N6	KX978539
A/Black-headed Gull/Netherlands/118/2012	H13N6	MF147977
A/Black-headed Gull/Netherlands/119/2012	H13N6	MF147208
A/Black-headed Gull/Netherlands/12/2012	H13N6	MF147771
A/Black-headed Gull/Netherlands/120/2012	H13N6	MF146637
A/Black-headed Gull/Netherlands/121/2012	H13N6	KX978308
A/Black-headed Gull/Netherlands/122/2012	H13N6	MF146479
A/Black-headed Gull/Netherlands/123/2012	H13N6	MF146534
A/Black-headed Gull/Netherlands/124/2012	H13N6	MF147740
A/Black-headed Gull/Netherlands/125/2012	H13N6	MF146435
A/Black-headed Gull/Netherlands/126/2012	H13N6	KX979088
A/Black-headed Gull/Netherlands/127/2012	H13N6	MF147719
A/Black-headed Gull/Netherlands/128/2012	H13N6	MF147553
A/Black-headed Gull/Netherlands/129/2012	H13N6	MF146833
A/Black-headed Gull/Netherlands/13/2012	H13N6	MF146523
A/Black-headed Gull/Netherlands/130/2012	H13N6	MF147007
A/Black-headed Gull/Netherlands/131/2012	H13N6	MF145735
A/Black-headed Gull/Netherlands/132/2012	H13N6	MF147533
A/Black-headed Gull/Netherlands/133/2012	H13N6	MF147558
A/Black-headed Gull/Netherlands/134/2012	H13N6	KX977630
A/Black-headed Gull/Netherlands/135/2012	H13N6	MF146431
A/Black-headed Gull/Netherlands/136/2012	H13N6	MF146500
A/Black-headed Gull/Netherlands/137/2012	H13N6	MF147405
A/Black-headed Gull/Netherlands/138/2012	H13N6	MF146583
A/Black-headed Gull/Netherlands/14/2012	H13N6	MF147101
A/Black-headed Gull/Netherlands/15/2012	H13N6	MF147814
A/Black-headed Gull/Netherlands/16/2012	H13N6	MF145897
A/Black-headed Gull/Netherlands/17/2012	H13N6	MF147189
A/Black-headed Gull/Netherlands/18/2012	H13N6	MF147647
A/Black-headed Gull/Netherlands/19/2012	H13N6	MF148130
A/Black-headed Gull/Netherlands/2/2012	H13N6	KX977772

Virus	Subtype	Accession number
A/Black-headed Gull/Netherlands/20/2012	H13N6	MF146063
A/Black-headed Gull/Netherlands/21/2012	H13N6	MF147878
A/Black-headed Gull/Netherlands/22/2012	H13N6	MF145799
A/Black-headed Gull/Netherlands/23/2012	H13N6	KX977887
A/Black-headed Gull/Netherlands/24/2012	H13N6	MF147679
A/Black-headed Gull/Netherlands/25/2012	H13N6	KX977702
A/Black-headed Gull/Netherlands/26/2012	H13N6	MF147342
A/Black-headed Gull/Netherlands/27/2012	H13N6	MF146953
A/Black-headed Gull/Netherlands/28/2012	H13N6	MF147705
A/Black-headed Gull/Netherlands/29/2012	H13N6	MF146506
A/Black-headed Gull/Netherlands/3/2012	H13N6	MF146473
A/Black-headed Gull/Netherlands/30/2012	H13N6	MF146800
A/Black-headed Gull/Netherlands/31/2012	H13N6	MF147302
A/Black-headed Gull/Netherlands/32/2012	H13N6	MF146858
A/Black-headed Gull/Netherlands/33/2012	H13N6	KX979200
A/Black-headed Gull/Netherlands/34/2012	H13N6	MF146167
A/Black-headed Gull/Netherlands/35/2012	H13N6	MF147197
A/Black-headed Gull/Netherlands/36/2012	H13N6	MF147122
A/Black-headed Gull/Netherlands/37/2012	H13N6	MF147911
A/Black-headed Gull/Netherlands/38/2012	H13N6	MF147940
A/Black-headed Gull/Netherlands/39/2012	H13N6	MF146672
A/Black-headed Gull/Netherlands/4/2012	H13N6	MF146383
A/Black-headed Gull/Netherlands/40/2012	H13N6	MF147673
A/Black-headed Gull/Netherlands/41/2012	H13N6	MF145921
A/Black-headed Gull/Netherlands/42/2012	H13N6	KX978470
A/Black-headed Gull/Netherlands/43/2012	H13N6	MF147698
A/Black-headed Gull/Netherlands/44/2012	H13N6	MF146594
A/Black-headed Gull/Netherlands/45/2012	H13N6	MF146496
A/Black-headed Gull/Netherlands/46/2012	H13N6	MF145843
A/Black-headed Gull/Netherlands/47/2012	H13N6	KX978071
A/Black-headed Gull/Netherlands/48/2012	H13N6	MF147537
A/Black-headed Gull/Netherlands/49/2012	H13N6	MF146211
A/Black-headed Gull/Netherlands/5/2012	H13N6	MF146395
A/Black-headed Gull/Netherlands/50/2012	H13N6	MF146634
A/Black-headed Gull/Netherlands/51/2012	H13N6	MF148145
A/Black-headed Gull/Netherlands/52/2012	H13N6	KX977670
A/Black-headed Gull/Netherlands/53/2012	H13N6	MF147610
A/Black-headed Gull/Netherlands/54/2012	H13N6	MF145811
A/Black-headed Gull/Netherlands/55/2012	H13N6	MF147656
A/Black-headed Gull/Netherlands/56/2012	H13N6	MF147422
A/Black-headed Gull/Netherlands/57/2012	H13N6	MF147944
A/Black-headed Gull/Netherlands/58/2012	H13N6	MF146043
A/Black-headed Gull/Netherlands/59/2012	H13N6	MF147717
A/Black-headed Gull/Netherlands/6/2012	H13N6	MF146975
A/Black-headed Gull/Netherlands/60/2012	H13N6	MF147750
A/Black-headed Gull/Netherlands/61/2012	H13N6	MF147841
A/Black-headed Gull/Netherlands/62/2012	H13N6	MF146710
A/Black-headed Gull/Netherlands/63/2012	H13N6	MF146214
A/Black-headed Gull/Netherlands/64/2012	H13N6	MF147469
A/Black-headed Gull/Netherlands/65/2012	H13N6	KX977811
A/Black-headed Gull/Netherlands/66/2012	H13N6	MF146872
A/Black-headed Gull/Netherlands/67/2012	H13N6	MF145901
A/Black-headed Gull/Netherlands/68/2012	H13N6	MF146441
A/Black-headed Gull/Netherlands/69/2012	H13N6	KX978831
A/Black-headed Gull/Netherlands/7/2012	H13N6	MF145876
A/Black-headed Gull/Netherlands/70/2012	H13N6	MF146204
A/Black-headed Gull/Netherlands/71/2012	H13N6	MF145709
A/Black-headed Gull/Netherlands/72/2012	H13N6	KX978101
A/Black-headed Gull/Netherlands/73/2012	H13N6	MF147965

Virus	Subtype	Accession number
A/Black-headed Gull/Netherlands/74/2012	H13N6	MF148047
A/Black-headed Gull/Netherlands/75/2012	H13N6	MF147095
A/Black-headed Gull/Netherlands/76/2012	H13N6	KX978834
A/Black-headed Gull/Netherlands/77/2012	H13N6	MF146562
A/Black-headed Gull/Netherlands/78/2012	H13N6	MF146693
A/Black-headed Gull/Netherlands/79/2012	H13N6	MF147375
A/Black-headed Gull/Netherlands/8/2012	H13N6	MF146964
A/Black-headed Gull/Netherlands/80/2012	H13N6	MF147078
A/Black-headed Gull/Netherlands/81/2012	H13N6	KX977728
A/Black-headed Gull/Netherlands/82/2012	H13N6	KX979079
A/Black-headed Gull/Netherlands/83/2012	H13N6	KX977618
A/Black-headed Gull/Netherlands/84/2012	H13N6	MF146502
A/Black-headed Gull/Netherlands/85/2012	H13N6	KX979040
A/Black-headed Gull/Netherlands/86/2012	H13N6	MF147754
A/Black-headed Gull/Netherlands/87/2012	H13N6	KX979045
A/Black-headed Gull/Netherlands/88/2012	H13N6	KX978218
A/Black-headed Gull/Netherlands/89/2012	H13N6	MF146309
A/Black-headed Gull/Netherlands/9/2012	H13N6	MF575308
A/Black-headed Gull/Netherlands/90/2012	H13N6	MF147768
A/Black-headed Gull/Netherlands/91/2012	H13N6	MF147484
A/Black-headed Gull/Netherlands/92/2012	H13N6	KX978027
A/Black-headed Gull/Netherlands/94/2012	H13N6	MF682816
A/Black-headed Gull/Netherlands/95/2012	H13N6	MF146344
A/Black-headed Gull/Netherlands/96/2012	H13N6	MF147573
A/Black-headed Gull/Netherlands/97/2012	H13N6	MF147559
A/Black-headed Gull/Netherlands/98/2012	H13N6	KX978937
A/Black-headed Gull/Netherlands/99/2012	H13N6	KX978911
A/Black-headed Gull/Republic of Georgia/2/2012	H13N6	CY185569
A/Duck/Hokkaido/W345/2012	H13N2	LC336769
A/Duck/Hokkaido/WZ68/2012	H13N2	AB812744
A/Gull/Massachusetts/12JR00671/2012	H13N6	CY195663
A/Mediterranean gull/Netherlands/1/2012	H13N6	MF147925
A/Ring-billed Gull/Massachusetts/12DC00060/2012	H13N6	CY195647
A/Ruddy Turnstone/New Jersey/AI12-1737/2012	H13N6	MH501657
A/Yellow-legged Gull/Georgia/4/2012	H13N8	MF147792
A/Yellow-legged Gull/Republic of Georgia/1/2012	mixed	CY185371
A/Yellow-legged Gull/Republic of Georgia/2/2012	H13N6	CY185601
A/Yellow-legged Gull/Republic of Georgia/3/2012	H13N6	CY185609
A/Yellow-legged Gull/Republic of Georgia/5/2012	H13N6	CY185673
A/Yellow-legged Gull/Republic of Georgia/6/2012	H13N6	CY185665
A/Black-headed Gull/Netherlands/10/2013	H13N8	KX977721
A/Black-headed Gull/Netherlands/6/2013	H13N8	MF146245
A/Black-headed Gull/Netherlands/7/2013	H13N8	KX977868
A/Black-headed Gull/Netherlands/8/2013	H13N8	MF147858
A/Black-headed Gull/Netherlands/9/2013	H13N8	MF146285
A/Glaucous-winged Gull/Alaska/387/2013	H13N2	KY131017
A/Glaucous-winged Gull/Alaska/410/2013	H13-mixed	KY131033
A/Glaucous-winged Gull/Alaska/414/2013	H13N2	KY131040
A/Glaucous-winged Gull/Alaska/458/2013	H13N2	KY131056
A/Glaucous-winged Gull/Alaska/544/2013	H13N2	KY131072
A/Glaucous-winged Gull/Alaska/545/2013	H13N2	KY131080
A/Glaucous-winged Gull/Alaska/654/2013	H13N2	KY131112
A/Glaucous-winged Gull/Alaska/660/2013	H13N2	KY131120
A/Glaucous-winged Gull/Alaska/664/2013	H13N2	KY131128
A/Glaucous-winged Gull/Alaska/667/2013	H13N2	KY131136
A/Glaucous-winged Gull/Alaska/695/2013	H13N2	KY131144
A/Gull/Massachusetts/13JR03320/2013	H13N6	CY195825
A/Ring-billed Gull/Massachusetts/13DC30736-1/2013	H13N8	CY195801
A/Ring-billed Gull/Massachusetts/13DC30736-2/2013	H13N8	CY195809

Virus	Subtype	Accession number
A/Yellow-legged Gull/Georgia/1/2013	H13N8	CY185625
A/Yellow-legged Gull/Georgia/2/2013	H13N8	CY185633
A/Black-headed Gull/Netherlands/1/2014	H13N2	KX978817
A/Black-headed Gull/Netherlands/10/2014	H13N6	KX979051
A/Black-headed Gull/Netherlands/11/2014	H13N6	KX977664
A/Black-headed Gull/Netherlands/12/2014	H13N2	KX978367
A/Black-headed Gull/Netherlands/13/2014	H13N2	MF147040
A/Black-headed Gull/Netherlands/14/2014	H13N6	MF147981
A/Black-headed Gull/Netherlands/15/2014	H13N6	KX978686
A/Black-headed Gull/Netherlands/16/2014	H13N6	MF146531
A/Black-headed Gull/Netherlands/17/2014	H13N6	KX979090
A/Black-headed Gull/Netherlands/2/2014	H13N2	MF145726
A/Black-headed Gull/Netherlands/23/2014	H13N2	KX978812
A/Black-headed Gull/Netherlands/24/2014	H13N2	MF147919
A/Black-headed Gull/Netherlands/25/2014	H13N2	KX978026
A/Black-headed Gull/Netherlands/26/2014	H13N6	KX978072
A/Black-headed Gull/Netherlands/27/2014	H13N2	KX977853
A/Black-headed Gull/Netherlands/28/2014	H13N6	KX979165
A/Black-headed Gull/Netherlands/29/2014	H13N6	KX978504
A/Black-headed Gull/Netherlands/3/2014	H13N6	KX977620
A/Black-headed Gull/Netherlands/30/2014	H13N6	MF146090
A/Black-headed Gull/Netherlands/31/2014	H13N6	MF575089
A/Black-headed Gull/Netherlands/32/2014	H13N6	KX978441
A/Black-headed Gull/Netherlands/33/2014	H13N6	MF575196
A/Black-headed Gull/Netherlands/34/2014	H13N6	MF575309
A/Black-headed Gull/Netherlands/35/2014	H13N6	KX978735
A/Black-headed Gull/Netherlands/37/2014	H13N2	MF148148
A/Black-headed Gull/Netherlands/4/2014	H13N2	MF575052
A/Black-headed Gull/Netherlands/5/2014	H13N6	KX978369
A/Black-headed Gull/Netherlands/6/2014	H13N2	KX978977
A/Black-headed Gull/Netherlands/7/2014	H13N6	MF147493
A/Black-headed Gull/Netherlands/8/2014	H13N2	KX978275
A/Black-headed Gull/Netherlands/9/2014	H13N2	MF146305
A/Eurasian curlew/Liaoning/ZH-186/2014	H13N6	KR010435
A/Eurasian curlew/Liaoning/ZH-385/2014	H13N8	KR010443
A/Ruddy Turnstone/New Jersey/UGAI14-1436/2014	H13N6	MH502664
A/Glaucous-winged Gull/Alaska/UGAI15-6732/2015	H13-mixed	KY131286
A/Glaucous-winged Gull/Alaska/UGAI15-6732/2015	H13-mixed	KY131287
A/Glaucous-winged Gull/Southcentral Alaska/15MB01429/2015	H13N6	CY213628
A/Glaucous-winged Gull/Southcentral Alaska/15MB01557/2015	H13N6	CY213620
A/Glaucous-winged Gull/Southcentral Alaska/15MB01610/2015	H13N6	CY213527
A/Glaucous-winged Gull/Southcentral Alaska/15MB01632/2015	H13N6	CY213636
A/Glaucous-winged Gull/Southcentral Alaska/15MB01645/2015	H13N6	CY213535
A/Glaucous-winged Gull/Southcentral Alaska/15MB01667/2015	H13-mixed	CY213644
A/Glaucous-winged Gull/Southcentral Alaska/15MB01693/2015	H13N6	CY213543
A/Glaucous-winged Gull/Southcentral Alaska/15MB01694/2015	H13N6	CY213663
A/Glaucous-winged Gull/Southcentral Alaska/15MB01776/2015	H13N6	CY213687
A/Glaucous-winged Gull/Southcentral Alaska/15MB02016/2015	H13N8	CY213695
A/Glaucous-winged Gull/Southcentral Alaska/15MB02018/2015	H13N8	CY213703
A/Gull/New Jersey/UGAI15-3767/2015	H13N3	MH501054
A/American oystercatcher/Chile/C20102/2016	H13N9	MH499091
A/Black skimmer/Chile/C20057/2016	H13N8	MH498778
A/Black skimmer/Chile/C20077/2016	H13N9	MH499127
A/Black skimmer/Chile/C20084/2016	H13N9	MH498752
A/Black skimmer/Chile/C20100/2016	H13N9	MH499241
A/Black skimmer/Chile/C20108/2016	H13N9	MH499144
A/Black skimmer/Chile/C20123/2016	H13N9	MH499019
A/Black skimmer/Chile/C20124/2016	H13N9	MH498871
A/Black skimmer/Chile/C20140/2016	H13N9	MH499102

Virus	Subtype	Accession number
A/Black skimmer/Chile/C20142/2016	H13N9	MH499225
A/Black-headed Gull/Netherlands/10/2016	H13N2	MF694241
A/Black-headed Gull/Netherlands/11/2016	H13N2	MF694057
A/Black-headed Gull/Netherlands/2/2016	H13N2	MF694026
A/Black-headed Gull/Netherlands/6/2016	H13N2	MF694199
A/Black-headed Gull/Netherlands/7/2016	H13N2	MF694207
A/Black-headed Gull/Netherlands/8/2016	H13N2	MF693954
A/Black-headed Gull/Netherlands/9/2016	H13N2	MF694046
A/Black-tailed Gull/Weihai/115/2016	H13N2	MF461180
A/Black-tailed Gull/Weihai/17/2016	H13N8	MF461188
A/Black-tailed Gull/Weihai/42/2016	H13N2	MH201562
A/Blackish oystercatcher/Chile/C20062/2016	H13N9	MH498730
A/Brown-hooded gull/Chile/C10246/2016	H13N2	MH499037
A/Elegant tern/Chile/C20085/2016	H13N9	MH499011
A/Elegant tern/Chile/C20093/2016	H13N9	MH499177
A/Franklin's gull/Chile/C17421/2016	H13N9	MH498978
A/Franklin's gull/Chile/C17422/2016	H13N9	MH499057
A/Franklin's gull/Chile/C20061/2016	H13N9	MH498647
A/Franklin's gull/Chile/C20069/2016	H13N9	MH498793
A/Franklin's gull/Chile/C20070/2016	H13N9	MH498930
A/Franklin's gull/Chile/C20086/2016	H13N9	MH499107
A/Franklin's gull/Chile/C20094/2016	H13N9	MH499041
A/Franklin's gull/Chile/C20110/2016	H13N9	MH499155
A/Franklin's gull/Chile/C20118/2016	H13N9	MH499132
A/Franklin's gull/Chile/C20149/2016	H13N9	MH499078
A/Franklin's gull/Chile/C20373/2016	H13N9	MH499160
A/Gull/Arica/71/2016	H13N2	MF099262
A/Kelp gull/Chile/C20137/2016	H13N9	MH498851
A/Kelp gull/Chile/C8594/2016	H13N2	MH499186
A/Kelp gull/Chile/C8595/2016	H13N2	MH498919
A/Kelp gull/Chile/C8599/2016	H13N2	MH499244
A/Kelp gull/Chile/C8602/2016	H13N2	MH498862
A/Kelp gull/Chile/C8609/2016	H13N2	MH498888
A/Kelp gull/Chile/C8939/2016	H13N2	MH498698
A/Sandpiper/Southcentral Alaska/16MB01145/2016	H13-mixed	CY213503
A/Shorebird/Chile/C7037/2016	H13N2	MH499147
A/Whimbrel/Chile/C20073/2016	H13N9	MH499218
A/Whimbrel/Chile/C20075/2016	H13N9	MH498668
A/Whimbrel/Chile/C20106/2016	H13N9	MH498656
A/Whimbrel/Chile/C20144/2016	H13N9	MH499009
A/Whimbrel/Chile/C20147/2016	H13N9	MH499072
A/White-backed stilt/Chile/C20090/2016	H13N9	MH498671
A/Kelp gull/Chile/C27733/2017	H13N8	MH499142
A/Laughing Gull/New Jersey/UGAI17-2839/2017	H13N6	MH068335
A/Laughing Gull/New Jersey/UGAI17-2843/2017	H13N6	MH068343
A/Laughing Gull/New Jersey/UGAI17-2850/2017	H13N6	MH068359
A/Laughing Gull/New Jersey/UGAI17-2856/2017	H13N6	MH068367

Table S3

Virus	Subtype	Accession number
A/Black-legged Kittiwake/Alaska/295/1975	H16N3	CY015160
A/Black-headed Gull/Turkmenistan/13/1976	H16N3	EU293864
A/Slender-billed Gull/Astrakhan/28/1976	H16N3	EU293865
A/Gull/MD/4985/79	**H16-mixed**	**KF612964**
A/LittleTern/Gurjev/779/1983	H16N3	EU148601
A/Mallard/Gurjev/785/1983	H16N3	EU148600
A/Fulica atra/Volga/635/1986	H16N3	EU564109
A/Herring Gull/NewJersey/780/1986	H16N3	CY136590
A/Shorebird/NewJersey/840/1986	H16N3	CY014599
A/Teal/Volga/671/1986	H16N3	EU148602
A/Herring Gull/DelawareBay/2617/1987	H16N3	CY136606
A/Laughing Gull/DelawareBay/2623/1987	H16N3	CY136614
A/Laughing Gull/DelawareBay/2839/1987	H16N3	CY136630
A/Herring Gull/Delaware/712/1988	H16N3	CY136729
A/Herring Gull/NJ/163/90	**H16-mixed**	**KF612962**
A/Gull/NJ/48/92	**H16-mixed**	**KF612961**
A/Laughing Gull/DelawareBay/296/1998	H16N3	CY127445
A/Black-headed Gull/Sweden/2/1999	H16N3	AY684888
A/Black-headed Gull/Sweden/3/1999	H16N3	AY684889
A/Black-headed Gull/Sweden/4/1999	H16N3	AY684890
A/Black-headed Gull/Sweden/5/1999	H16N3	AY684891
A/Herring Gull/NewYork/AI00-532/2000	H16N3	CY144178
A/Waterfowl/GA/96623-7/01	**H16N3**	**KF612963**
A/Gull/Denmark/68110/2002	H16N3	GQ247872
A/Black-headed Gull/Sweden/9476/2005	**H16N3**	**KR087605**
A/Black-headed Gull/Sweden/9478/2005	**H16N3**	**MK027211**
A/Black-headed Gull/Sweden/9479/2005	**H16N3**	**MK027212**
A/Black-headed Gull/Sweden/9492/2005	**H16N3**	**KR087606**
A/Black-headed Gull/Sweden/9502/2005	**H16N3**	**KR087607**
A/Black-headed Gull/Sweden/9504/2005	**H16N3**	**KR087608**
A/Herring Gull/Finland/13022/2005	H16	KX455114
A/LittleTern/Sweden/8897/2005	**H16N3**	**KR087604**
A/wildbird/Sweden/1/2005	H16N3	KR087602
A/wildbird/Sweden/2/2005	H16N3	KR087603
A/Black-headed Gull/Mongolia/1756/2006	H16N3	GQ907294
A/Common Gull/Norway/101617/2006	H16N3	FM179755
A/Environment/Alaska/NWRC184854-12/2006	H16N3	CY122507
A/Environment/NewHampshire/NWRC182016-06/2006	H16N3	CY122448
A/Environment/RhodeIsland/NWRC182872-06/2006	H16N3	CY122476
A/Environment/Utah/NWRC184989-18/2006	H16N3	CY122509
A/European Herring Gull/Netherlands/5/2006	H16N3	MF147450
A/Glaucous Gull/Alaska/44198-027/2006	H16N3	HM059998
A/Herring Gull/Norway/101623/2006	H16N3	FM179756
A/Little Tern/Sweden/55316/2006	H16N3	KR087616
A/Shorebird/Delaware/168/2006	H16N3	EU030976
A/Shorebird/Delaware/172/2006	H16N3	CY130110
A/Shorebird/Delaware/195/2006	H16N3	CY045383
A/Black-headed Gull/Netherlands/1/2007	**H16N3**	**KR087609**
A/Black-headed Gull/Netherlands/3/2007	**H16N3**	**KR087610**
A/Black-headed Gull/Netherlands/5/2007	**H16N3**	**KR087611**
A/Black-headed Gull/Netherlands/7/2007	H16N3	KX978760
A/Black-headed Gull/Netherlands/8/2007	**H16N3**	**KR087612**
A/Black-headed Gull/Netherlands/9/2007	**H16N3**	**KR087613**
A/Environment/CO/492008/07	**H16N3**	**KF612965**
A/Black-headed Gull/Netherlands/100/2008	H16N3	MF147121
A/Black-headed Gull/Netherlands/101/2008	H16N3	KX978252
A/Black-headed Gull/Netherlands/26/2008	H16N3	MF147314
A/Black-headed Gull/Netherlands/27/2008	H16N3	MF146654
A/Black-headed Gull/Netherlands/28/2008	H16N3	MF146238

Virus	Subtype	Accession number
A/Black-headed Gull/Netherlands/29/2008	H16N3	MF146193
A/Black-headed Gull/Netherlands/33/2008	H16N3	MF148040
A/Black-headed Gull/Netherlands/36/2008	H16N3	MF147982
A/Black-headed Gull/Netherlands/40/2008	H16N3	MF146828
A/Black-headed Gull/Netherlands/41/2008	H16N3	MF146643
A/Black-headed Gull/Netherlands/42/2008	H16N3	MF145764
A/Black-headed Gull/Netherlands/43/2008	H16N8	MF147250
A/Black-headed Gull/Netherlands/45/2008	H16N3	MF146076
A/Black-headed Gull/Netherlands/46/2008	H16N3	MF146816
A/Black-headed Gull/Netherlands/47/2008	H16N3	MF146537
A/Black-headed Gull/Netherlands/50/2008	H16N3	MF145888
A/Black-headed Gull/Netherlands/53/2008	H16N3	MF146769
A/Black-headed Gull/Netherlands/54/2008	H16N3	MF147728
A/Black-headed Gull/Netherlands/56/2008	H16N3	MF146014
A/Black-headed Gull/Netherlands/57/2008	H16N3	MF146085
A/Black-headed Gull/Netherlands/59/2008	H16N3	MF147239
A/Black-headed Gull/Netherlands/60/2008	H16N3	MF146436
A/Black-headed Gull/Netherlands/61/2008	H16N3	MF682709
A/Black-headed Gull/Netherlands/62/2008	H16N3	MF147547
A/Black-headed Gull/Netherlands/63/2008	H16N3	KX979453
A/Black-headed Gull/Netherlands/69/2008	H16N3	MF145725
A/Black-headed Gull/Netherlands/72/2008	H16N3	MF146541
A/Black-headed Gull/Netherlands/75/2008	H16N3	MF147192
A/Black-headed Gull/Netherlands/76/2008	H16N3	MF146737
A/Black-headed Gull/Netherlands/77/2008	H16N3	MF146931
A/Black-headed Gull/Netherlands/78/2008	H16N3	MF146836
A/Black-headed Gull/Netherlands/79/2008	H16N3	MF147849
A/Black-headed Gull/Netherlands/80/2008	H16N3	MF146060
A/Black-headed Gull/Netherlands/81/2008	H16N3	KX977730
A/Black-headed Gull/Netherlands/82/2008	H16N3	MF146767
A/Black-headed Gull/Netherlands/84/2008	H16N3	MF147070
A/Black-headed Gull/Netherlands/85/2008	H16N3	MF146577
A/Black-headed Gull/Netherlands/89/2008	H16N3	MF148111
A/Black-headed Gull/Netherlands/91/2008	H16N3	MF147657
A/Black-headed Gull/Netherlands/92/2008	H16N3	KX978323
A/Black-headed Gull/Netherlands/98/2008	H16N3	KX979456
A/Black-headed Gull/Netherlands/99/2008	H16N3	KX978955
A/Glaucous-winged Gull/Southcentral Alaska/12NH01265/2008	H16N3	CY196748
A/Glaucous-winged Gull/Southcentral Alaska/12NH01285/2008	H16N3	CY196756
A/Glaucous-winged Gull/Southcentral Alaska/12NH01518/2008	H16N3	CY196764
A/Glaucous-winged Gull/Southcentral Alaska/12NH01538/2008	H16N3	CY196772
A/Glaucous-winged Gull/Southcentral Alaska/12NH01540/2008	H16N3	CY196780
A/Glaucous-winged Gull/Southcentral Alaska/12NH01593/2008	H16N3	CY196788
A/Glaucous-winged Gull/Southcentral Alaska/12NH01600/2008	H16N3	CY196796
A/Glaucous-winged Gull/Southcentral Alaska/12NH01647/2008	H16N3	CY196804
A/Glaucous-winged Gull/Southcentral Alaska/12NH01679/2008	H16N3	CY196812
A/Great black-backed Gull/Netherlands/1/2008	H16N3	MF146805
A/Black-headed Gull/Netherlands/10/2009	**H16N3**	**KR087614**
A/Black-headed Gull/Netherlands/12/2009	H16N3	MF147927
A/Black-headed Gull/Netherlands/14/2009	H16N3	KX978997
A/Black-headed Gull/Netherlands/15/2009	H16N3	MF146410
A/Black-headed Gull/Netherlands/16/2009	H16N3	KX978083
A/Black-headed Gull/Netherlands/19/2009	H16N3	MF146148
A/Black-headed Gull/Netherlands/21/2009	**H16N3**	**KR087615**
A/Black-headed Gull/Netherlands/22/2009	H16N3	KX978709
A/Black-headed Gull/Netherlands/23/2009	H16N3	MF146268
A/Black-headed Gull/Netherlands/24/2009	H16N3	MF103719
A/Black-headed Gull/Netherlands/25/2009	H16N3	MF147715
A/Black-headed Gull/Netherlands/26/2009	**H16N3**	**KR087572**

Virus	Subtype	Accession number
A/Black-headed Gull/Netherlands/28/2009	H16N3	KX977785
A/Black-headed Gull/Netherlands/32/2009	H16N3	KX979111
A/Black-headed Gull/Netherlands/35/2009	H16N3	KX979094
A/Mallard/Quebec/02916-1/2009	H16	CY125606
A/Black-headed Gull/Iceland/713/2010	H16N3	CY138145
A/Black-headed Gull/Netherlands/11/2010	H16N3	KX977650
A/Black-headed Gull/Netherlands/14/2010	H16N3	KX977766
A/Black-headed Gull/Netherlands/3/2010	H16N3	KX978612
A/Black-headed Gull/Netherlands/9/2010	H16N3	KX978651
A/European Herring Gull/Netherlands/1/2010	H16N3	KX979488
A/Glaucous-winged Gull/Southcentral Alaska/10JR01814/2010	H16N3	CY196009
A/Glaucous-winged Gull/SoutheasternAlaska/10JR01572R0/2010	H16N3	CY130493
A/Glaucous-winged Gull/SoutheasternAlaska/10JR01681R0/2010	H16N3	CY130501
A/Glaucous-winged Gull/SoutheasternAlaska/10JR01700R0/2010	H16N3	CY130509
A/Glaucous-winged Gull/SoutheasternAlaska/10JR01811R0/2010	H16N3	CY130517
A/Gull/SoutheasternAlaska/10JR01527R0/2010	H16N3	CY130485
A/Herring Gull/Newfoundland/GR032/2010	H16N3	KC845043
A/Black-headed Gull/Netherlands/1/2011	H16N3	KX978434
A/Black-headed Gull/Netherlands/11/2011	H16N3	MF147513
A/Black-headed Gull/Netherlands/13/2011	H16N3	KX978398
A/Black-headed Gull/Netherlands/22/2011	H16N3	MF575026
A/Black-headed Gull/Netherlands/27/2011	H16N3	MF146675
A/Black-headed Gull/Netherlands/28/2011	H16N3	MF146920
A/Black-headed Gull/Netherlands/29/2011	H16N3	KX979432
A/Black-headed Gull/Netherlands/30/2011	H16N3	MF145738
A/Black-headed Gull/Netherlands/31/2011	H16N3	MF147961
A/Black-headed Gull/Netherlands/32/2011	H16N3	KX979603
A/Black-headed Gull/Netherlands/33/2011	H16N3	MF146352
A/Black-headed Gull/Netherlands/34/2011	H16N3	MF147682
A/Black-headed Gull/Netherlands/37/2011	H16N3	MF146714
A/Black-headed Gull/Netherlands/6/2011	H16N3	MF146111
A/Black-headed Gull/Netherlands/7/2011	H16N3	MF575109
A/Glaucous-winged Gull/Southcentral Alaska/11JR00366/2011	H16N3	CY196403
A/Glaucous-winged Gull/Southcentral Alaska/11JR01368/2011	H16N3	CY196411
A/Glaucous-winged Gull/Southcentral Alaska/11JR01710/2011	H16N3	CY196419
A/Glaucous-winged Gull/Southcentral Alaska/11JR01711/2011	H16N3	CY196427
A/Glaucous-winged Gull/Southcentral Alaska/11JR01712/2011	H16N3	CY196435
A/Glaucous-winged Gull/Southcentral Alaska/11JR01713/2011	H16N3	CY196443
A/Glaucous-winged Gull/Southcentral Alaska/11JR01716/2011	H16N3	CY196451
A/Glaucous-winged Gull/Southcentral Alaska/11JR01719/2011	H16N3	CY196459
A/Glaucous-winged Gull/Southcentral Alaska/11JR01722/2011	H16-mixed	CY195614
A/Glaucous-winged Gull/Southcentral Alaska/11JR01724/2011	H16N3	CY196467
A/Glaucous-winged Gull/Southcentral Alaska/11JR01725/2011	H16N3	CY196475
A/Glaucous-winged Gull/Southcentral Alaska/11JR01732/2011	H16N3	CY196483
A/Glaucous-winged Gull/Southcentral Alaska/11JR01733/2011	H16N3	CY196491
A/Glaucous-winged Gull/Southcentral Alaska/11JR01734/2011	H16N3	CY196499
A/Glaucous-winged Gull/Southcentral Alaska/11JR01736/2011	H16N3	CY196507
A/Glaucous-winged Gull/Southcentral Alaska/11JR01738/2011	H16N3	CY196515
A/Glaucous-winged Gull/Southcentral Alaska/11JR01739/2011	H16N3	CY196523
A/Glaucous-winged Gull/Southcentral Alaska/11JR01761/2011	H16N3	CY196531
A/Glaucous-winged Gull/Southcentral Alaska/11JR01785/2011	H16N3	CY196539
A/Glaucous-winged Gull/Southcentral Alaska/11JR01852/2011	H16N3	CY196547
A/Glaucous-winged Gull/Southcentral Alaska/11JR01859/2011	H16N3	CY196555
A/Glaucous-winged Gull/Southcentral Alaska/11JR01871/2011	H16N3	CY196563
A/Glaucous-winged Gull/Southcentral Alaska/11JR01902/2011	H16N3	CY196571
A/Glaucous-winged Gull/Southcentral Alaska/11JR01906/2011	H16N3	CY196579
A/Glaucous-winged Gull/Southcentral Alaska/11JR01908/2011	H16N3	CY196587
A/Glaucous-winged Gull/Southcentral Alaska/11JR02017/2011	H16N3	CY196595
A/Glaucous-winged Gull/Southcentral Alaska/11JR02272/2011	H16N3	CY196603

Virus	Subtype	Accession number
A/Black-headed Gull/Netherlands/107/2012	H16N3	KX978749
A/Black-headed Gull/Netherlands/114/2012	H16N3	KX977892
A/Black-headed Gull/Netherlands/93/2012	H16N3	KX979159
A/Black-headed Gull/Republic of Georgia/4/2012	H16N3	CY185585
A/Environment/California/1242V/2012	H16N3	CY176997
A/Glaucous-winged Gull/Southcentral Alaska/12MB01573/2012	H16N3	CY195671
A/Glaucous-winged Gull/Southcentral Alaska/12MB01577/2012	H16N3	CY195679
A/Glaucous-winged Gull/Southcentral Alaska/12MB01618/2012	H16N3	CY195687
A/Glaucous-winged Gull/Southcentral Alaska/12MB01620/2012	H16N3	CY195695
A/Glaucous-winged Gull/Southcentral Alaska/12MB01812/2012	H16N3	CY195703
A/Glaucous-winged Gull/Southcentral Alaska/12MB01823/2012	H16N3	CY195711
A/Glaucous-winged Gull/Southcentral Alaska/12NH01263/2012	H16N3	CY195785
A/Glaucous-winged Gull/Southcentral Alaska/12NH01632/2012	H16N3	CY195793
A/Gull/Massachusetts/12JR00662/2012	H16N3	CY195655
A/Black-headed Gull/Netherlands/2/2013	H16N3	KX977836
A/Black-headed Gull/Netherlands/3/2013	H16N3	KX978186
A/Black-headed Gull/Netherlands/4/2013	H16N3	KX979151
A/Black-headed Gull/Netherlands/5/2013	H16N3	KX978693
A/California Gull/California/1196P/2013	H16N3	CY177441
A/Duck/Hokkaido/WZ82/2013	H16N3	AB937721
A/Duck/Hokkaido/WZ82/2013	H16N3	LC339707
A/Glaucous-winged Gull/Alaska/567/2013	H16N3	KY131088
A/Glaucous-winged Gull/Southcentral Alaska/13MB01431/2013	H16N3	CY239288
A/Glaucous-winged Gull/Southcentral Alaska/13MB02410/2013	H16N3	CY239296
A/Glaucous-winged Gull/Southcentral Alaska/13MB02526/2013	H16N3	CY213711
A/Glaucous-winged Gull/Southcentral Alaska/13MB02527/2013	H16N3	CY213719
A/Glaucous-winged Gull/Southcentral Alaska/13MB02558/2013	H16N3	CY213727
A/Glaucous-winged Gull/Southcentral Alaska/13MB02561/2013	H16N3	CY213735
A/Glaucous-winged Gull/Southcentral Alaska/13MB02569/2013	H16N3	CY213743
A/Glaucous-winged Gull/Southcentral Alaska/13MB02582/2013	H16N3	CY213751
A/Glaucous-winged Gull/Southcentral Alaska/13MB02593/2013	H16N3	CY213759
A/Glaucous-winged Gull/Southcentral Alaska/13MB02599/2013	H16N3	CY213767
A/Gull/Massachusetts/13WP00522/2013	H16N3	CY195833
A/Gull/Massachusetts/13WP00539/2013	H16N3	CY195841
A/Laughing Gull/New Jersey/AI13-1937/2013	H16N3	MH501070
A/Ruddy Turnstone/New Jersey/AI13-2872/2013	H16N3	MH502494
A/Black-headed Gull/Netherlands/18/2014	H16N3	MF146608
A/Black-headed Gull/Netherlands/36/2014	H16N3	MF575190
A/European Herring Gull/Netherlands/2/2014	H16N3	MF147061
A/Glaucous-winged Gull/Alaska/915/2014	H16N3	KT338609
A/Glaucous-winged Gull/Southcentral Alaska/14MB00623/2014	H16N3	CY206862
A/Glaucous-winged Gull/Southcentral Alaska/14MB01306/2014	H16N3	CY206870
A/Glaucous-winged Gull/Southcentral Alaska/14MB01318/2014	H16N3	CY206878
A/Glaucous-winged Gull/Southcentral Alaska/14MB01336/2014	H16N3	CY206677
A/Glaucous-winged Gull/Southcentral Alaska/14MB01383/2014	H16N3	CY206886
A/Glaucous-winged Gull/Southcentral Alaska/14MB01392/2014	H16N3	CY206894
A/Glaucous-winged Gull/Southcentral Alaska/14MB01417/2014	H16N3	CY206902
A/Glaucous-winged Gull/Southcentral Alaska/14MB01418/2014	H16N3	CY206910
A/Glaucous-winged Gull/Southcentral Alaska/14MB01422/2014	H16N3	CY206918
A/Glaucous-winged Gull/Southcentral Alaska/14MB01438/2014	H16N3	CY206926
A/Glaucous-winged Gull/Southcentral Alaska/14MB01444/2014	H16N3	CY239304
A/Glaucous-winged Gull/Southcentral Alaska/14MB01615/2014	H16N3	CY206934
A/Glaucous-winged Gull/Southcentral Alaska/14MB01705/2014	H16N3	CY206942
A/Glaucous-winged Gull/Southcentral Alaska/14MB01770/2014	H16N3	CY206950
A/Glaucous-winged Gull/Southcentral Alaska/14MB01819/2014	H16N3	CY206958
A/Glaucous-winged Gull/Southcentral Alaska/14MB01884/2014	H16N3	CY206966
A/Glaucous-winged Gull/Southcentral Alaska/14MB01886/2014	H16N3	CY206974
A/Glaucous-winged Gull/Southcentral Alaska/14MB01893/2014	H16N3	CY206982
A/Glaucous-winged Gull/Southcentral Alaska/14MB01926/2014	H16N3	CY206990

Virus	Subtype	Accession number
A/Glaucous-winged Gull/Southcentral Alaska/14MB01959/2014	H16N3	CY206998
A/Glaucous-winged Gull/Southcentral Alaska/14MB02018/2014	H16N3	CY207006
A/Glaucous-winged Gull/Southcentral Alaska/14MB02081/2014	H16N3	CY207014
A/Glaucous-winged Gull/Southcentral Alaska/14MB02094/2014	H16N3	CY239312
A/Mallard/Alaska/903/2014	H16N3	KT338601
A/Northern pintail/Alaska/886/2014	H16N3	KT338585
A/Black-headed Gull/Netherlands/1/2015	H16N3	MF147650
A/Black-headed Gull/Netherlands/2/2015	H16N3	KX978663
A/Black-headed Gull/Netherlands/3/2015	H16N3	KX978028
A/Black-headed Gull/Netherlands/4/2015	H16N3	KX978525
A/Black-headed Gull/Netherlands/5/2015	H16N3	KX977739
A/European Herring Gull/Netherlands/3/2015	H16N3	MF693968
A/Glaucous-winged Gull/Southcentral Alaska/15MB01680/2015	H16N3	CY213655
A/Glaucous-winged Gull/Southcentral Alaska/15MB01735/2015	H16N3	CY213551
A/Glaucous-winged Gull/Southcentral Alaska/15MB01747/2015	H16N3	CY213671
A/Glaucous-winged Gull/Southcentral Alaska/15MB01758/2015	H16N3	CY213679
A/Gull/New Jersey/UGAI15-3414/2015	H16N3	MH501022
A/Gull/New Jersey/UGAI15-3459/2015	H16N3	MH501038
A/Lesser black-backed Gull/Netherlands/1/2015	H16N3	MF694110
A/Black-headed Gull/Netherlands/1/2016	H16N3	MF694134
A/Black-headed Gull/Netherlands/3/2016	H16N3	MF694124
A/Brown-hooded gull/Chile/C8851/2016	H16N3	MH498904
A/Environment/New Jersey/UGAI16-0787/2016	H16N3	CY240828
A/Environment/New Jersey/UGAI16-0887/2016	H16-mixed	CY240896
A/Environment/New Jersey/UGAI16-1048/2016	H16N3	CY240948
A/Environment/New Jersey/UGAI16-1713/2016	H16N3	CY241634
A/Franklin's gull/Chile/C10784/2016	H16N3	MH134702
A/Franklin's gull/Chile/C10794/2016	H16N3	MH134685
A/Glaucous-winged Gull/Southcentral Alaska/16MB00031/2016	H16N3	CY239376
A/Glaucous-winged Gull/Southcentral Alaska/16MB00033/2016	H16N3	CY239269
A/Glaucous-winged Gull/Southcentral Alaska/16MB02936/2016	H16N3	CY239320
A/Glaucous-winged Gull/Southcentral Alaska/16MB02941/2016	H16N3	CY239328
A/Glaucous-winged Gull/Southcentral Alaska/16MB02942/2016	H16N3	CY239336
A/Glaucous-winged Gull/Southcentral Alaska/16MB02960/2016	H16N3	CY239344
A/Glaucous-winged Gull/Southcentral Alaska/16MB03027/2016	H16N3	CY239352
A/Glaucous-winged Gull/Southcentral Alaska/16MB03039/2016	H16N3	CY239360
A/Glaucous-winged Gull/Southcentral Alaska/16MB03046/2016	H16N3	CY239368
A/Glaucous-winged Gull/Southcentral Alaska/16MB03089/2016	H16N3	CY239384
A/Glaucous-winged Gull/Southcentral Alaska/16MB03160/2016	H16N3	CY239392
A/Sandpiper/Southcentral Alaska/16MB01145/2016	H16-mixed	CY213504

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: Virtual [redacted] Site-Visit
Date: Wednesday, April 22, 2020 10:12:33 AM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

Yes, great. We don't need [redacted]
My number is [redacted] Give me your number is you want me to call you.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
CC: [redacted]
[redacted]**:** RE: Virtua[redacted] Site-Visit

[redacted]

I am flexible this afternoon/evening, except for a call at 6 pm Central (but that's probably too late for you anyway). If [redacted] should be included, we'd have to do it tomorrow (early morning US, noon/early afternoon Europe, evening in Japan).

Shall we have a call today at 8:30 NL time (without [redacted]), and follow up with him tomorrow, if needed?

Best,

[redacted]

From: [redacted]>
Sent: Wednesday, April 22, 2020 9:59 AM
To: [redacted]>
Cc: [redacted]

Subject: Re: Virtual [redacted] Site-Visit

[redacted]
Do you have time for a quick chat tonight about the slides? I could do any time after 8:30 pm [redacted] time.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]
[redacted]
[redacted]
[redacted] **:** RE: Virtual [redacted] Site-Visit

[redacted] and [redacted]

Attached please find a few summary slides for the [redacted] that you could use for your summary presentations … but that's up to you, of course.
Please let me know if you have any questions about the slides.
Please also let me know if you'd like me to prepare other slides.

Thanks,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]
Date: Tuesday, April 28, 2020 7:51:53 AM

Thanks [redacted], for your very quick responses!

[redacted]

On Tue, Apr 28, 2020 at 1:49 PM [redacted] wrote:

> I did click the link!
>
> [redacted]
>
> ---
>
> **From:** [redacted]>
> **Sent:** Tuesday, April 28, 2020 9:48 PM
> **To:** [redacted]
> **Cc:** [redacted]
> **Subject:** RE: [redacted]
>
> Dear All,
>
> We have a SARS-CoV-2 call coming up at the top of the hour with [redacted] presenting today – so, he may not respond. I just read through it real quick and added a couple of comments (please feel free to ignore them if they don't make any sense).
>
> Thanks,
>
> [redacted]

From: [redacted]
Sent: Tuesday, April 28, 2020 6:55 AM
To: [redacted]
Cc: [redacted]
Subject: [redacted]

[redacted]

Attached a Word document a two page preliminary application to the [redacted] scheme. We'd mentioned this scheme as an alternate funding source when we were talking about alternate funding a couple of months ago, and [redacted], this is the scheme for which [redacted] has collected your CVs and other info over the last few days.

If you have any suggestions or comments on the two-page scientific proposal attached, if you could get them to us by about 4pm [redacted] time today that would be great, it has to be submitted at 5pm [redacted] time today.

Please use track changes so we can see any changes. If you are comfortable with google docs, you can make the changes (in suggestion mode please) or add comments directly in our master document here [redacted]

[redacted], the only thing we'll need you to do is to click on a link in an email you'll get in the next hour agreeing to be a co-applicant.

[redacted], thanks for sending the CV info you've already sent. [redacted] and [redacted] have extracted from that most of what is needed, and will enter everything they can into the [redacted] system on your behalf. [redacted] has drafted some "supporting academic history" that [redacted] require, please check that or provide something different if you prefer.

The rest of this email is not needed to read now, it is some background info on the [redacted] and

whether this is the right approach to them or not.

[redacted] primarily funds in the [redacted], and developing world collaborators with [redacted] groups. Thus not our type of collaboration. Nevertheless there is one award they do, the "collaborative award" which we're putting in the 2-pager preliminary application today.

Getting past the preliminary stage of the award we are applying for now has not been hard for us in the past, I think the bar is fairly low, but might be higher given COVID. Being granted an award however seems difficult We were the lead applicant one of these collaborative awards about 7 years ago, on [redacted], passed the preliminary stage, wrote a full proposal, but did not get the award. We were a co-applicant on another, on ancient viruses, that that was funded only after a third attempt. We've also looked at all of the looking at how many of these are funded each year, (and even fewer with [redacted] based collaborators).

I'm planning to speak with [redacted], the director of the [redacted] about our best approach. Perhaps a partnership with [redacted] is possible. Or perhaps he'll suggest backing off from going after one of these collaborative awards and for us in [redacted] to just apply for their much easier to get [redacted]-centric awards just to our group in [redacted]. If he indicates the latter, we might have to go that route as our funding runs out in [redacted] when [redacted] runs out, and a smaller local [redacted] award would provide stop gap until the [redacted] money flows again. Also, this [redacted] Collaborative award is substantially less than our [redacted] awards, it would only partially fund our current efforts.

Given the COVID situation however, I don't expect to get to talk with him for long or more than once. So I figure it is best to see what [redacted] have to say in the coming days before that call with [redacted]. In the meantime, we want to submit this preliminary application on for the [redacted] so we are eligible for this scheme with you as partners in case he indicates it should be our approach.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]
Date: Friday, May 22, 2020 1:26:45 PM

Ugh. That is a shame. And useless comments back, as usual.
[redacted]

From: [redacted]
Sent: Friday, May 22, 2020 2:44 PM
To: [redacted]
Cc: [redacted]
Subject: Fwd: [redacted]

We don't get to write a full proposal to [redacted]. I'm surprised.

Thanks all for your help pulling the preliminary proposal together.

[redacted]

---------- Forwarded message ---------
From: [redacted]
Date: Fri, May 22, 2020 at 12:28 PM
Subject: [redacted]
To: [redacted] >

Dear [redacted]

Reference number: [redacted]

Thank you for your recent preliminary application for a [redacted]

We have now considered the preliminary applications for the current competition and I am sorry to tell you that your proposal was not shortlisted for further consideration. The applications were assessed for a number of criteria, including the strength of the research question; the articulated need for a collaborative approach and the track records of the applicants.

There was a great deal of interest in the scheme and a large number of high quality applications were received. I regret that, when viewed in competition with the other applications, your submission was not chosen to go forward for further consideration.

I realise that this decision will come as a disappointment and hope that you will be able to obtain support from elsewhere. I would be grateful if you could convey this decision to the other applicants.

If you have any questions, please do not hesitate to contact me

Yours sincerely

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: more FW: RE: Rick Bright
Date: Thursday, April 23, 2020 4:04:12 AM
Attachments: image001.png
image002.png
image003.png

Very interesting.

> 2020/04/23 15:46 [redacted]:
>
> thanks [redacted]
>
> On Thu, Apr 23, 2020 at 7:28 AM [redacted] wrote:
>
> > Jeremy Diamond tweeted Rick's written comment (email):
> >
> > https://twitter.com/JDiamond1/status/1253056646802214912
> >
> > Yours sincerely,
> >
> > [redacted]
> >
> > [redacted]
> >
> > [redacted]
> >
> > ---
> >
> > **Van:** [redacted]
> > **CC:** [redacted]
> > [redacted] Re: more FW: RE: Rick Bright
> >
> > wow
> >
> > good for him for making it public!
> >
> > On Wed, Apr 22, 2020 at 11:05 PM [redacted] > wrote:
> >
> > > More on Rick.

From: [redacted]
Sent: Wednesday, April 22, 2020 4:41 PM
To: [redacted]
Subject: RE: Rick Bright

With no further comments.

https://www.cnn.com/2020/04/22/politics/rick-bright-barda-trump-coronavirus/index.html

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: hamster model
Date: Thursday, May 14, 2020 5:06:00 PM
Attachments: [redacted]ki Hamster JVI 18 e01693-17.full.pdf
[redacted] Transmission of SARS-CoV-2 in Domestic Cats.pdf

Dear [redacted]

It was nice talking to you.
Please see our manuscript describing hamsters as an animal model for COVID-19 uploaded at:

https://uwmadison.box.com/s/srvv0awja3mdiorinbkz11zgq24990ne

Three hamster papers by other groups are available on the internet. The data are slightly different.
Also, we have a hamster airborne-transmission model in influenza viruses (see attached). We have not done this with SARS-CoV-2 yet, but it would be straightforward to just use SARS-CoV-2 instead of influenza virus.
Lastly, we just published a paper on cat-to-cat transmission of SARS-CoV-2 (attached). But, I do not think cats are a good model for the experiments we discussed.

Best,
[redacted]

From: [redacted]
Sent: Monday, May 11, 2020 8:53 PM
To: [redacted]
Cc: [redacted]
Subject: RE:

H[redacted], I 'll be glad to chat. Lets see if [redacted] can find a time. Hope your doing well. [redacted]

From: [redacted]
Sent: Monday, May 11, 2020 5:49 AM
To: [redacted]
Cc: [redacted]

Subject:

Dear [redacted],

Are you collaborating with anyone to analyze mutant SARS-CoV-2 strains?
We have a hamster model running and could test any mutants you may have or plan to create. I have some ideas, but I am sure you already thought about them.

Best,

[redacted]

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PATHOGENESIS AND IMMUNITY

Syrian Hamster as an Animal Model for the Study of Human Influenza Virus Infection

Kiyoko Iwatsuki-Horimoto,[a] Noriko Nakajima,[b] Yurie Ichiko,[a] Yuko Sakai-Tagawa,[a] Takeshi Noda,[c] Hideki Hasegawa,[b] Yoshihiro Kawaoka[a,d,e]

[a]Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan

[b]Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

[c]Laboratory of Ultrastructural Virology, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

[d]Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin—Madison, Madison, Wisconsin, USA

[e]Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan

ABSTRACT Ferrets and mice are frequently used as animal models for influenza research. However, ferrets are demanding in terms of housing space and handling, whereas mice are not naturally susceptible to infection with human influenza A or B viruses. Therefore, prior adaptation of human viruses is required for their use in mice. In addition, there are no mouse-adapted variants of the recent H3N2 viruses, because these viruses do not replicate well in mice. In this study, we investigated the susceptibility of Syrian hamsters to influenza viruses with a view to using the hamster model as an alternative to the mouse model. We found that hamsters are sensitive to influenza viruses, including the recent H3N2 viruses, without adaptation. Although the hamsters did not show weight loss or clinical signs of H3N2 virus infection, we observed pathogenic effects in the respiratory tracts of the infected animals. All of the H3N2 viruses tested replicated in the respiratory organs of the hamsters, and some of them were detected in the nasal washes of infected animals. Moreover, a 2009 pandemic (pdm09) virus and a seasonal H1N1 virus, as well as one of the two H3N2 viruses, but not a type B virus, were transmissible by the airborne route in these hamsters. Hamsters thus have the potential to be a small-animal model for the study of influenza virus infection, including studies of the pathogenicity of H3N2 viruses and other strains, as well as for use in H1N1 virus transmission studies.

IMPORTANCE We found that Syrian hamsters are susceptible to human influenza viruses, including the recent H3N2 viruses, without adaptation. We also found that a pdm09 virus and a seasonal H1N1 virus, as well as one of the H3N2 viruses, but not a type B virus tested, are transmitted by the airborne route in these hamsters. Syrian hamsters thus have the potential to be used as a small-animal model for the study of human influenza viruses.

KEYWORDS animal model, hamster, influenza

Influenza A viruses are known to have a broad host range. They can infect not only humans but also waterfowl, poultry, sea mammals, pigs, horses, cats, dogs, and other species (1). Ferrets are used as an experimental animal model for studies of influenza virus infection because they are naturally susceptible to influenza A and B viruses, and their clinical features and the pathological changes associated with the bronchitis and pneumonia that they experience resemble those that occur in humans (2–5). Ferrets

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have also been used for studies of influenza virus transmission (6–8). However, ferrets demand considerable housing space and can be difficult to handle. Mice are frequently used as an animal model for influenza research. However, mice are not naturally susceptible to human influenza A or B viruses, with the exception of highly pathogenic human H5N1 viruses (9), the reconstructed 1918 pandemic influenza virus (10), the A(H1N1) pandemic 2009 [A(H1N1)pdm09] virus (6, 11), and H7N9 viruses (8, 12–14). Therefore, prior adaptation of human viruses is required for their experimental use in mice. Other rodents, such as rats, guinea pigs, and cotton rats, are also occasionally used as animal models. To use rats experimentally, rat-adapted viruses, which induce a mild form of the disease with no mortality, are required (15). Guinea pigs have been used as a model of transmission of influenza viruses (16); however, even though the H5N1 and 1918 pandemic viruses replicated in the lungs and nasal turbinates of these animals, no weight loss or morbidity was observed (17). Recently, cotton rats have been considered a potential animal model for influenza viruses (18). They are susceptible to both human influenza A and B viruses without prior adaptation (19–22), and an H5N1 virus was shown to be lethal in this species (22). However, cotton rats are not widely available. Thus, each animal model has limitations or drawbacks.

Current animal models for influenza virus studies have one additional limitation. Historically, H3N2 viruses, such as A/Hong Kong/1/68, A/Aichi/2/68, and A/Guizhou/54/89, were adapted to mice, and the mouse-adapted variants were used for numerous studies (23–26). However, the recent H3N2 viruses cannot replicate in mice (27), which prevents mouse models from being used to test the activities of therapeutic or prophylactic drugs against the currently prevalent viruses.

Previously, hamsters were proposed to be a model animal for the study of influenza, because of their sensitivity to human isolates (28–33) and contact transmission of human isolates (34). Hamsters showed sensitivity equivalent to that of ferrets and guinea pigs (33). Moreover, in a vaccine efficacy study, hamsters differentially recognized a single amino acid difference involving egg adaptation in the H1 hemagglutinin (HA) protein (33). In this study, we investigated the susceptibility of Syrian hamsters to influenza viruses to assess the possibility of using these animals as a small-animal model for influenza research.

RESULTS

Detection of sialyloligosaccharides in the respiratory tract of hamsters. First, we examined the sialyloligosaccharide distribution in the respiratory tract of 4- and 8-week-old female hamsters. The nasal epithelial cell populations varied at different locations (35, 36). At the distal section of the nasal cavity of 4-week-old hamsters, squamous epithelial and respiratory epithelial cells predominated (Fig. 1A). The population of olfactory epithelial cells gradually increased from the middle to the deep section of the nasal cavity (Fig. 1B and C), such that there were ultimately more olfactory epithelial cells than respiratory epithelial cells in the deep portion of the nasal cavity (Fig. 1D). *Sambucus nigra* lectin I (SNA I), which is specific for sialic acid linked to galactose by an α-2,6 linkage (SAα2,6Gal), mainly reacted with the respiratory epithelial cells in the distal section of the nasal cavity (Fig. 1A to C); in contrast, *Maackia amurensis* lectin II (MAA II), which is specific for sialic acid linked to galactose by an α-2,3 linkage (SAα2,3Gal), mainly reacted with the olfactory epithelial cells in the proximal portion of the nasal turbinates of the hamsters (Fig. 1C and D). In the pharynx, trachea, and bronchus, both SNA I and MAA II strongly reacted with the epithelial cells (Fig. 2A to C). In contrast, only MAA II strongly reacted with the epithelial cells in the lungs (Fig. 2D). Similar findings were obtained with the 8-week-old hamsters (data not shown). These results indicate that 4- and 8-week-old hamsters have appreciable amounts of SAα2,6Gal in the distal end of their nasal turbinates and SAα2,3Gal in their lungs.

Growth properties of H3N2 viruses in hamsters and mice. Four- or 8-week-old female hamsters and 6-week-old female BALB/c or DBA/2 mice were anesthetized and intranasally inoculated with 1.0×10^6 PFU of A/Tokyo/IMS6-1/2013 (H3N2/2013), A/Tokyo/IMS2-1/2014 (H3N2/2014), or A/Tokyo/UT-HP002/2016 (H3N2/2016) virus ($n =$

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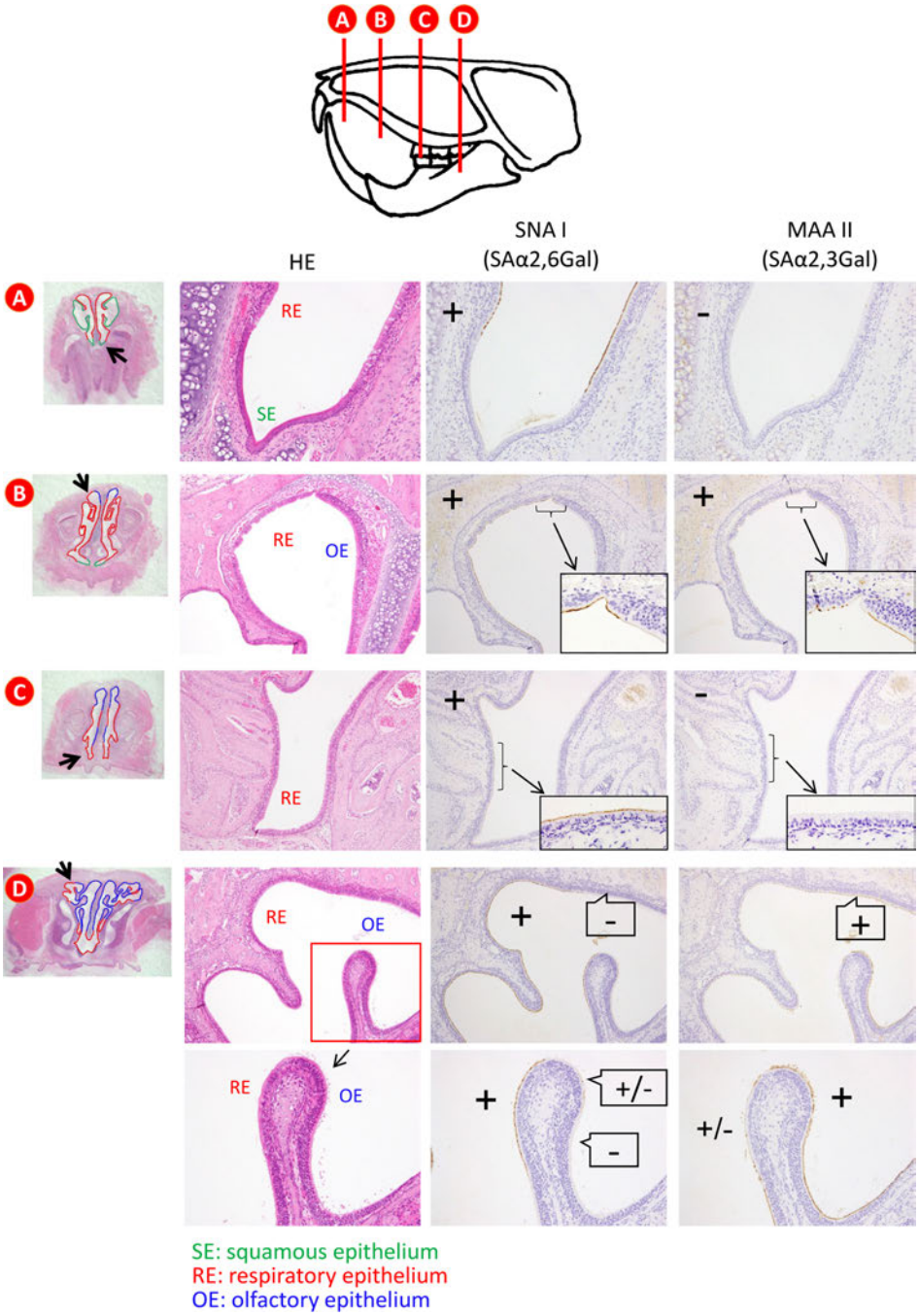

FIG 1 Detection of SAα2,6Gal and SAα2,3Gal oligosaccharides in the nasal turbinate by using lectins. Sections of a 4-week-old Syrian hamster were reacted with SNA I and MAA II. The vertical lines of the image at the top indicate the anterior surfaces of transverse tissue blocks (A to D). (A) A distal section of the nasal cavity of a 4-week-old hamster showing the predominance of squamous epithelial cells and respiratory epithelial cells. (B to D) The population of olfactory epithelial cells gradually increased from the middle to the deep section of the nasal cavity (B, C); more olfactory epithelial cells than respiratory epithelial cells were present in the deep portion of the nasal cavity (D). SNA I, which is specific for SAα2,6Gal, mainly reacted with respiratory epithelial cells in the distal section of the nasal cavity (A to C); in contrast, MAA II, which is specific for SAα2,3Gal, mainly reacted with olfactory epithelial cells in the proximal portion of the nasal turbinates of hamsters (C, D). HE, hematoxylin and eosin staining.

9 hamsters and $n = 13$ mice for each virus). The clinical condition and body weight of 3 hamsters and 4 mice infected with each virus were assessed daily, and nasal wash specimens were collected from the hamsters every other day for virus titration. None of the infected animals showed any clinical signs (data not shown) or weight loss, with

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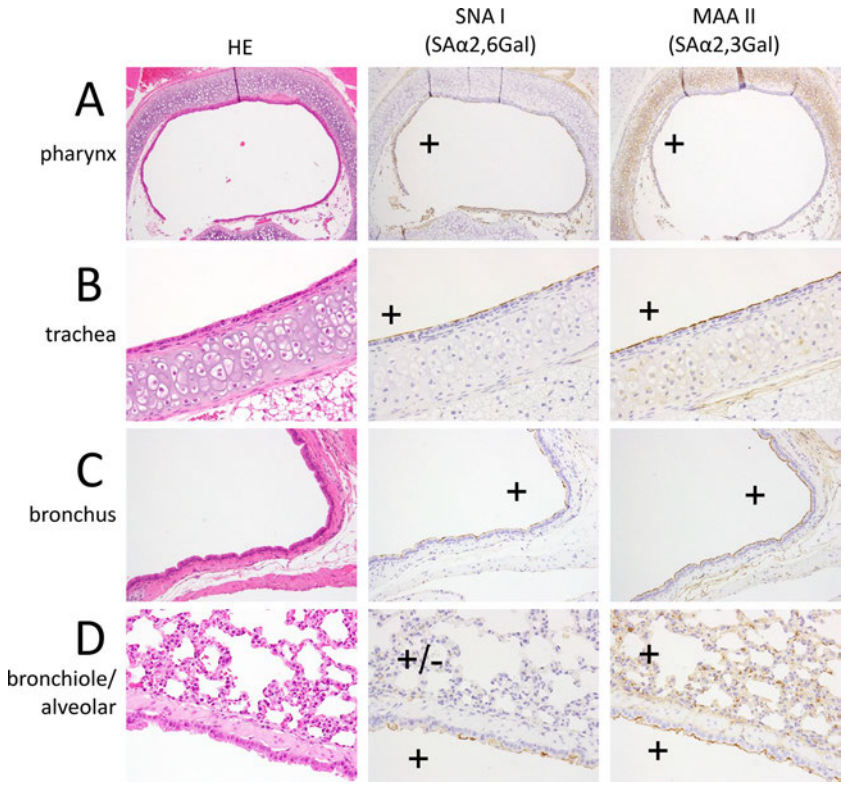

FIG 2 Detection of SAα2,6Gal and SAα2,3Gal oligosaccharides in the pharynx (A), trachea (B), bronchus (C), and bronchiole/alveolar region (D) of a 4-week-old Syrian hamster. In the pharynx, trachea, and bronchus, both SNA I and MAA II strongly reacted with the epithelial cells (A, B, C). In contrast, MAA II strongly reacted with the epithelial cells in the lungs (D). HE, hematoxylin and eosin staining.

the exception of a slight decrease in the body weight of the H3N2/2016-infected mice (Fig. 3A). Although the H3N2/2013 virus was not detected in the nasal washes of H3N2/2013-infected hamsters, viruses were detected in the nasal washes until day 4 in all H3N2/2014-infected hamsters and 5 of 6 H3N2/2016-infected hamsters (Fig. 4A). On days 1 ($n = 3$, mice only), 3 ($n = 3$), and 6 ($n = 3$) postinfection, animals were euthanized and their organs were collected for virological and pathological examination. Although no clinical signs were observed, all of the viruses replicated in the respiratory organs of the hamsters (Table 1). In contrast, among the DBA/2 mice, the H3N2/2013 virus was found in the trachea of only one mouse. H3N2/2014 virus titers were moderate in the nasal turbinates on days 1 and 3, and the virus was found in the lung of one DBA/2 mouse and the trachea of another DBA/2 mouse on day 1 (Table 1). The H3N2/2016 virus was detected at low levels in the lungs, tracheas, and nasal turbinates of DBA/2 mice. Virus titers in BALB/c mice were generally lower than those in DBA/2 mice, as previously reported (37). No virus was detected in any DBA/2 or BALB/c mouse on day 6 (Table 1). These results indicate that hamsters are more susceptible to the recent H3N2 viruses than are BALB/c or DBA/2 mice.

Growth properties of pdmH1N1 viruses in hamsters and mice. We performed experiments with pandemic H1N1 (pdmH1N1) viruses similar to those performed for H3N2 viruses and described above. Both the pandemic A/Hiroshima/19/2013 (pdmH1N1/2013) and pandemic A/Tokyo/IMS1-1/2014 (pdmH1N1/2014) viruses were pathogenic in mice. Specifically, all of the infected DBA/2 mice died during the observation period (Fig. 3B), and although none of the infected BALB/c mice died, their body weights decreased (Fig. 3B) and virus titers were very high in their respiratory tracts (Table 2). Hamsters were also highly susceptible to the pdmH1N1 viruses. Although neither the 4-week-old hamsters nor the 8-week-old hamsters infected with pdmH1N1/2013 virus showed any body weight loss (Fig. 3B, left), we did observe a

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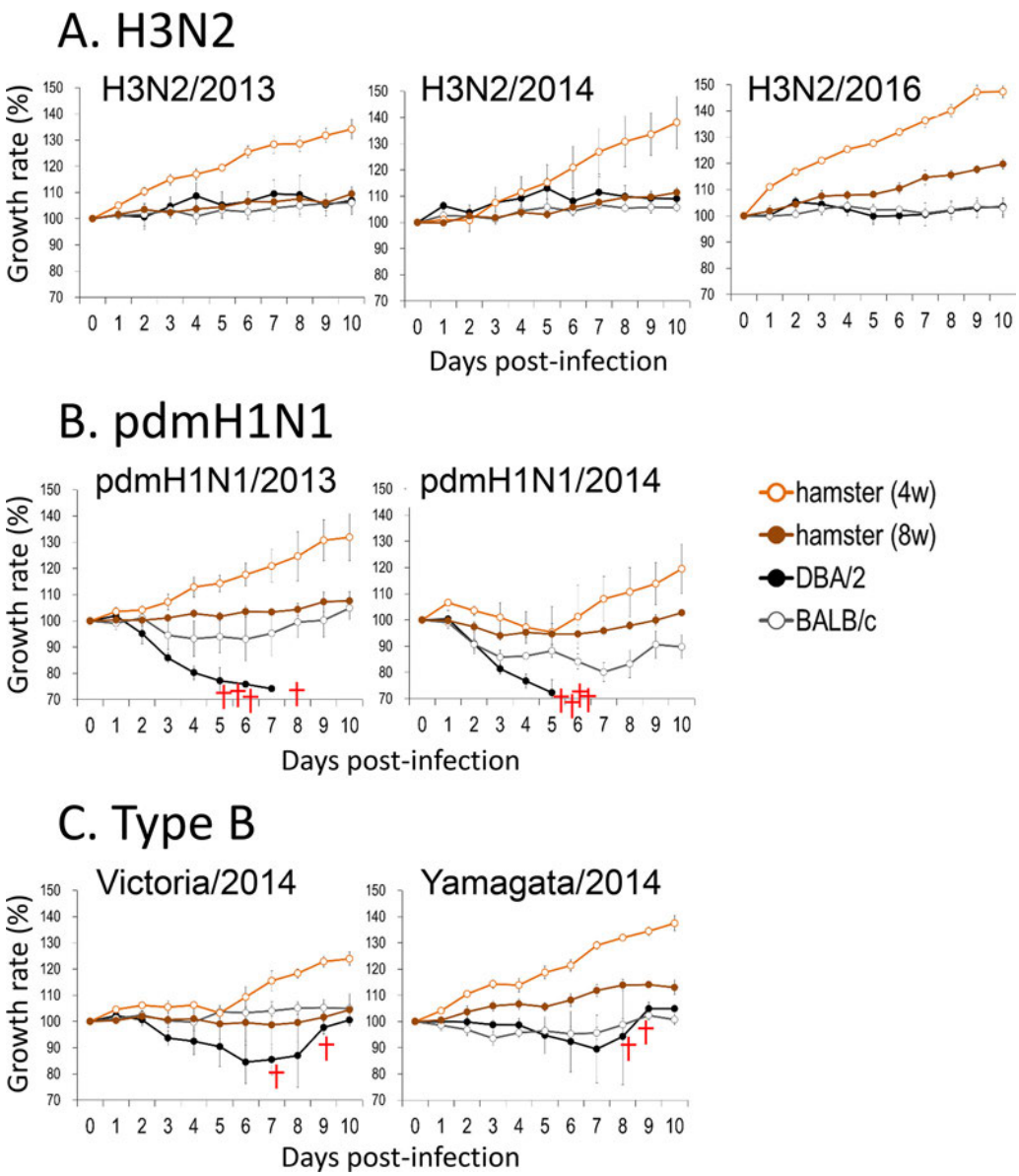

FIG 3 Body weight changes in infected animals. Six-week-old female BALB/c mice and DBA/2 mice and 4- or 8-week-old female Syrian hamsters were anesthetized and intranasally inoculated with 10^6 PFU of H3N2/2013 (A, left), H3N2/2014 (A, middle), H3N2/2016 (A, right), pdmH1N1/2013 (B, left), pdmH1N1/2014 (B, right), Victoria/2014 (C, left), or Yamagata/2014 (C, right) virus ($n = 3$ hamsters and $n = 4$ mice for each virus). The body weights of individual animals inoculated with viruses are depicted as a percentage of the body weight compared with that on day 0. Crosses indicate dead infected animals.

decrease in the body weights of the 4- and 8-week-old hamsters infected with pdmH1N1/2014 virus (Fig. 3B, right). Virus was detected in the nasal washes of all of the pdmH1N1/2013- and pdmH1N1/2014-infected hamsters at least until day 4 (Fig. 4B). In addition, high titers of both the pdmH1N1/2013 and pdmH1N1/2014 viruses were detected in the respiratory tracts of the hamsters, especially on day 3 (Table 2). These results indicate that hamsters are highly susceptible to pdmH1N1 viruses.

Growth properties of type B viruses in hamsters and mice. We further tested the susceptibility of mice and hamsters to influenza B viruses as described above for the H3N2 and pdmH1N1 viruses. Both B/Kamakura/8/2014 (Victoria lineage; Victoria/2014) and B/Kamakura/10/2014 (Yamagata lineage; Yamagata/2014) were highly pathogenic in DBA/2 mice. Two of 4 DBA/2 mice infected with each of the viruses died during the observation period (Fig. 3C), but none of the infected BALB/c mice died from their type

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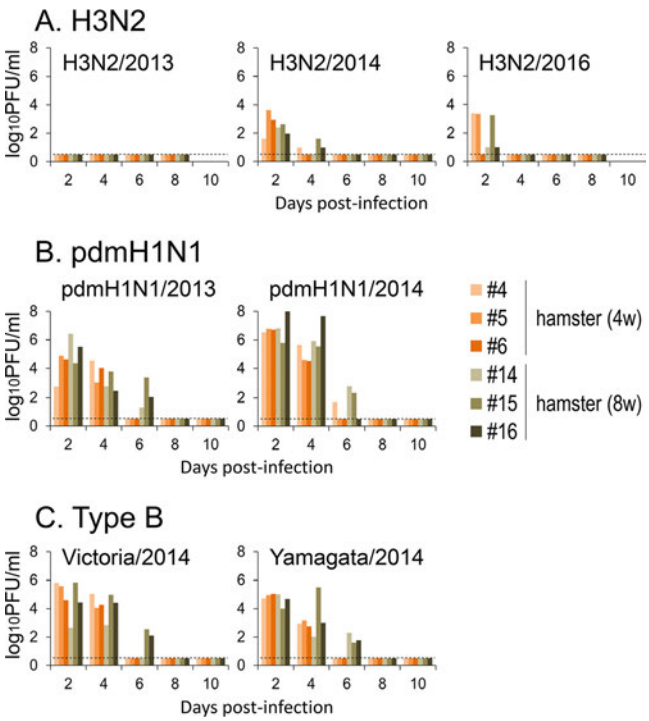

FIG 4 Virus titers in the nasal washes of 4-week-old or 8-week-old hamsters infected with 10^6 PFU of H3N2/2013 (A, left), H3N2/2014 (A, middle), H3N2/2016 (A, right), pdmH1N1/2013 (B, left), pdmH1N1/2014 (B, right), Victoria/2014 (C, left), or Yamagata/2014 (C, right) virus ($n = 3$ hamsters for each virus). Nasal wash specimens with 400 μl of PBS from each hamster were collected every other day for virus titration. Virus titers were determined by using a plaque assay on MDCK cells. The lower limit of detection is indicated by the horizontal dashed lines.

B virus infection. Body weight loss was observed among the BALB/c mice after Yamagata/2014 infection but not after Victoria/2014 infection (Fig. 3C). In contrast, Victoria/2014 infection blocked the body weight gain of both the 4- and 8-week-old hamsters (Fig. 3C). Although the titers in the Victoria/2014- and the Yamagata/2014-infected hamsters were lower than those in the pdmH1N1 virus-infected hamsters, virus was detected in the nasal washes of both the Victoria/2014- and the Yamagata/2014-infected hamsters at least until day 4 (Fig. 4C). Similarly, although the titers in the respiratory tracts of the type B virus-infected mice were lower than those of the pdmH1N1 viruses, appreciably high Victoria/2014 and Yamagata/2014 virus titers were detected in the respiratory tracts of both the DBA/2 and BALB/c mice (Tables 2 and 3). Also in hamsters, although some variations were found depending on the individual animals, the viruses used, and the age of the animals, both type B viruses replicated appreciably well in the respiratory organs. These results indicate that hamsters are also susceptible to type B viruses.

Pathological analyses of influenza virus-infected animals. Four-week-old female hamsters and 6-week-old female BALB/c mice or DBA/2 mice were infected with H3N2/2013 ($n = 4$), pdmH1N1/2013 ($n = 4$), or Victoria/2014 ($n = 4$) virus. On days 3 ($n = 2$) and 6 ($n = 2$) postinfection, the animals were euthanized and their organs were collected for pathological examinations. The pdmH1N1/2013 virus-infected DBA/2 mice scheduled for sampling on day 6 died on day 3 and on day 6. The DBA/2 mouse that died on day 6 was dissected just after death for pathological analyses.

The number of antigen-positive cells detected by immunohistochemistry (Table 4) showed a pattern similar to that of the virus titers (Tables 1 to 3). In the case of H3N2/2013 virus infection, virus antigens were not detected in any DBA/2 or BALB/c mouse but were detected in the nasal turbinate of one hamster and in the trachea and bronchus of another hamster (Table 4). There were few antigen-positive cells, and it was

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TABLE 1 Virus titers in tissues of animals infected with H3N2 viruses[a]

Animal (age) and organ[b]	Titer (log_{10} PFU/g)																										
	H3N2/2013									H3N2/2014									H3N2/2016								
	24 hpi			Day 3			Day 6			24 hpi			Day 3			Day 6			24 hpi			Day 3			Day 6		
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28	29
DBA/2 mice																											
Nasal turb	—[c]	—	—	—	—	—	—	—	—	3.4	4.9	3.6	4.0	3.8	3.6	—	—	—	2.1	—	—	—	—	—	—	—	—
Trachea	2.3	—	—	—	—	—	—	—	—	—	—	2.4	—	—	—	—	—	—	2.3	3.7	3.6	—	—	—	—	—	—
Lung	—	—	—	—	—	—	—	—	—	1.9	—	—	—	—	—	—	—	—	3.3	3.7	1.8	—	—	—	—	—	—
BALB/c mice																											
Nasal turb	—	—	—	—	—	—	—	—	—	3.0	3.7	—	3.8	2.8	4.9	—	—	—	—	—	—	—	—	—	—	—	—
Trachea	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.6	—	—	—	—	—	—	—
Lung	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.9	—	—	—	—	—	—
Hamsters (4 wk)																											
Nasal turb	NA[d]	NA	NA	4.0	4.5	5.7	—	—	2.6	NA	NA	NA	5.7	5.3	5.9	2.8	—	2.8	NA	NA	NA	6.0	5.7	5.8	—	—	—
Trachea	NA	NA	NA	5.6	5.0	4.6	—	—	—	NA	NA	NA	4.0	3.0	4.5	—	—	—	NA	NA	NA	3.7	4.5	3.4	—	—	—
Lung																											
R cra/acce	NA	NA	NA	5.2	3.4	—	—	—	—	NA	NA	NA	2.7	—	—	—	—	—	NA	NA	NA	4.9	4.6	—	—	—	—
R middle	NA	NA	NA	4.7	4.0	—	—	—	—	NA	NA	NA	3.4	—	—	—	—	—	NA	NA	NA	4.7	5.4	—	—	—	—
R caudal	NA	NA	NA	5.3	3.5	—	—	—	—	NA	NA	NA	2.9	2.5	—	—	—	—	NA	NA	NA	5.8	6.1	—	—	—	—
L	NA	NA	NA	5.5	4.3	—	—	—	—	NA	NA	NA	2.9	2.2	—	—	—	—	NA	NA	NA	6.3	4.6	—	—	—	—
Hamsters (8 wk)																											
Nasal turb	NA	NA	NA	4.1	4.0	4.7	—	2.6	—	—	NA	NA	6.0	5.5	5.5	2.5	—	—	—	NA	NA	6.3	6.1	5.7	—	—	—
Trachea	NA	NA	NA	3.6	5.0	4.0	—	—	—	—	NA	NA	4.5	4.5	4.5	—	—	—	—	NA	NA	4.9	3.6	3.3	—	—	—
Lung																											
R cra/acce	NA	NA	NA	—	3.0	—	—	—	—	NA	NA	NA	5.1	—	—	—	—	—	NA	NA	NA	5.3	6.0	3.9	—	—	—
R middle	NA	NA	NA	2.7	3.8	—	—	—	—	NA	NA	NA	5.4	3.7	—	—	—	1.9	NA	NA	NA	6.0	—	—	—	—	—
R caudal	NA	NA	NA	3.1	4.4	—	—	—	—	NA	NA	NA	5.1	5.1	—	—	—	—	NA	NA	NA	2.0	—	—	—	—	—
L	NA	NA	NA	4.3	6.2	—	—	—	—	NA	NA	NA	5.1	2.2	—	—	—	—	NA	NA	NA	—	—	3.2	—	—	—

[a]Six-week-old female DBA/2 mice and BALB/c mice and 4- or 8-week-old female Syrian hamsters were anesthetized and intranasally inoculated with 10^6 PFU of the H3N2/2013, H3N2/2014, or H3N2/2016 virus. Three animals per group were euthanized at 24 h postinfection (hpi) (mice only) and on days 3 and 6 postinfection.
[b]Nasal turb, nasal turbinate; R cra/acce, right cranial and accessory lobes; R middle, right middle lobe; R caudal, right caudal lobe; L, left lobe.
[c]—, virus not detected.
[d]NA, not available.

difficult to determine the cell tropism of the H3N2 viruses. Histopathological changes were limited in the nasal turbinate of H3N2/2013-infected hamsters, and virus antigens were mainly detected in the olfactory epithelia (Fig. 5). In contrast, in the cases of pdmH1N1/2013 or Victoria/2014 virus infection, virus antigens were detected in the respiratory organs of all animals infected with either pdmH1N1/2013 virus or Victoria/2014 virus (Table 4). In the nasal turbinate of the infected hamsters, inflammatory cells infiltrated the lamina propria (Fig. 5). The olfactory epithelia of the pdmH1N1/2013-infected hamsters were partially eroded (Fig. 5). There were fewer antigen-positive cells in the hamsters than in the mice (Table 4). The distribution of virus antigens in the nasal turbinate differed between type A (H3N2 and pdmH1N1/2013)- and type B (Victoria/2014)-infected hamsters. In the nasal turbinate of the H3N2- or pdmH1N1/2013-infected hamsters, virus antigens were detected mainly in the olfactory epithelia rather than in the respiratory epithelia (Fig. 5). In contrast, in the nasal turbinate of the Victoria/2014-infected hamsters, virus antigens were detected in both the respiratory epithelia and the olfactory epithelia (Fig. 5).

Transmissibility of influenza viruses in hamsters. To assess the transmissibility of influenza viruses in hamsters, two animals each infected with 10^6 PFU of A/Texas/50/2012 (H3N2; TX50), H3N2/2014, A/California/04/2009 (pdmH1N1; CA04), A/Brisbane/59/2007 (H1N1; BNE59), or B/Yokohama/UT-K1A/2011 (type B Victoria linage; UTK1A) were placed in the larger room of a transmission cage, and on the next day, a naive hamster was placed in the adjacent smaller room of the cage (Fig. 6A). Three sets of hamsters (nine animals in total) were used for each virus. We recovered viruses from the nasal washes of all infected hamsters, except for three animals infected with TX50 (Fig. 6C to

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TABLE 2 Virus titers in tissues of animals infected with pdmH1N1 viruses[a]

Animal (age) and organ[b]	Titer (log_{10} PFU/g)																	
	pdmH1N1/2013									pdmH1N1/2014								
	24 hpi			Day 3			Day 6			24 hpi			Day 3			Day 6		
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19
DBA/2 mice																		
Nasal turb	8.3	8.3	8.2	7.5	8.3	7.1	ND[c]	ND	8.2	9.7	8.4	8.2	8.1	7.9	8.2	ND	ND	ND
Trachea	8.5	8.9	8.8	7.1	7.1	7.3	ND	ND	7.3	8.1	7.6	8.2	7.2	7.5	7.4	ND	ND	ND
Lung	8.5	8.6	8.4	7.1	7.2	7.4	ND	ND	7.2	9.0	9.0	9.0	7.7	8.2	8.1	ND	ND	ND
BALB/c mice																		
Nasal turb	8.1	8.8	8.1	7.4	7.3	7.5	7.3	7.1	7.1	8.4	8.5	8.5	8.1	9.0	7.9	7.0	6.5	6.4
Trachea	7.9	8.3	8.1	6.6	6.0	6.1	5.3	6.2	6.3	6.9	7.4	7.3	5.9	5.9	5.3	5.3	5.8	4.1
Lung	8.3	8.3	8.5	7.1	7.2	6.7	5.4	5.6	5.5	8.9	8.6	8.6	7.3	7.4	6.5	4.6	4.3	4.3
Hamsters (4 wk)																		
Nasal turb	NA[d]	NA	NA	8.2	8.5	7.8	2.8	2.8	3.4	NA	NA	NA	8.8	8.7	9.0	2.9	3.1	4.5
Trachea	NA	NA	NA	8.1	7.7	8.0	—[e]	—	2.3	NA	NA	NA	8.4	8.9	8.5	—	—	4.4
Lung																		
R cra/acce	NA	NA	NA	8.2	6.5	—	—	2.8	3.0	NA	NA	NA	8.2	6.5	7.5	2.1	2.9	1.8
R middle	NA	NA	NA	6.3	6.4	—	—	1.9	3.3	NA	NA	NA	6.9	7.7	6.7	—	5.8	5.8
R caudal	NA	NA	NA	6.3	6.4	—	—	2.9	4.7	NA	NA	NA	8.2	7.2	8.1	3.4	4.9	2.7
L	NA	NA	NA	7.4	6.1	—	—	1.8	2.9	NA	NA	NA	7.1	7.6	7.4	—	4.4	—
Hamsters (8 wk)																		
Nasal turb	NA	NA	NA	8.0	7.5	7.5	5.4	5.5	5.7	—	NA	NA	8.7	8.9	8.5	3.7	3.4	4.7
Trachea	NA	NA	NA	7.4	6.2	7.1	2.5	2.4	1.8	—	NA	NA	8.5	8.5	8.2	2.4	2.3	2.3
Lung																		
R cra/acce	NA	NA	NA	5.3	7.0	7.2	3.3	—	—	NA	NA	NA	7.2	7.4	7.6	4.6	—	—
R middle	NA	NA	NA	6.2	7.3	7.0	1.9	—	6.3	NA	NA	NA	7.5	7.1	6.8	3.0	5.9	3.6
R caudal	NA	NA	NA	6.8	7.8	7.0	5.3	—	1.6	NA	NA	NA	7.4	7.3	7.3	3.3	1.6	—
L	NA	NA	NA	7.0	6.7	6.9	5.3	2.2	1.9	NA	NA	NA	7.2	7.3	7.1	4.6	2.4	2.0

[a]Six-week-old female DBA/2 mice and BALB/c mice and 4- or 8-week-old female Syrian hamsters were anesthetized and intranasally inoculated with 10^6 PFU of pdmH1N1/2013 or pdmH1N1/2014 virus. Three animals per group were euthanized at 24 h postinfection (hpi) (mice only) and on days 3 and 6 postinfection.
[b]Nasal turb, nasal turbinate; R cra/acce, right cranial and accessory lobes; R middle, right middle lobe; R caudal, right caudal lobe; L, left lobe.
[c]ND, not done. These animals died on day 5 (DBA/2 mice 7, 17, 18, and 19) or day 6 (DBA/2 mouse 8).
[d]NA, not available.
[e]—, virus not detected.

G, left). No virus was detected in the nasal washes of all three hamsters that were exposed to hamsters infected with TX50 (H3N2) or UTK1A (type B) (Fig. 6C and G). In contrast, all of the hamsters that were exposed to hamsters infected with CA04 (pdmH1N1) (Fig. 6E), two of three hamsters (pairs 1 and 2) that were exposed to hamsters infected with BNE59 (H1N1) (Fig. 6F), and one of three hamsters (pair 3) that were exposed to hamsters infected with H3N2/2014(H3N2) (Fig. 6D) shed viruses. Serum antibody titers against each virus confirmed infection of the animals from which virus was recovered, whereas the exposed hamsters from which virus was not recovered did not seroconvert, with the exception of hamsters exposed to hamsters infected with H3N2/2014(H3N2) (data not shown); all three hamsters exposed to the H3N2/2014(H3N2)-infected group seroconverted, with the virus neutralization titers being 1:16 for pair 1, 1:256 for pair 2, and 1:128 for pair 3, indicating that this virus transmitted to all three hamsters. These results indicate that hamsters can be used to evaluate the airborne transmissibility of human influenza viruses.

DISCUSSION

In this study, we demonstrated that hamsters are susceptible to influenza viruses, including the recent H3N2 viruses. Although hamsters did not show weight loss or clinical signs of H3N2 virus infection, we detected virus antigens in the respiratory tracts of infected hamsters without adaptation of the viruses. Hamsters are easier to handle than ferrets, and recent H3N2 viruses do not appreciably replicate in mice; therefore, these findings indicate that hamsters may represent an alternative rodent model for studies of recent human influenza viruses, especially H3N2 viruses.

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TABLE 3 Virus titers in tissues of animals infected with influenza B viruses[a]

Animal (age) and organ[b]	Titer (log_{10} PFU/g)																	
	Victoria/2014									Yamagata/2014								
	24 hpi			Day 3			Day 6			24 hpi			Day 3			Day 6		
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19
DBA/2 mice																		
Nasal turb	6.7	6.5	6.6	6.0	6.1	6.0	4.8	5.2	4.1	5.8	5.3	6.1	5.4	5.2	3.9	2.6	3.1	2.6
Trachea	6.9	7.1	6.6	5.1	5.9	5.3	4.7	4.8	3.4	5.2	4.2	4.5	2.8	4.2	—[c]	4.2	5.0	—
Lung	6.4	6.4	6.6	6.2	6.1	6.2	5.2	5.9	4.7	4.7	4.7	3.6	3.0	4.4	3.4	2.3	2.5	—
BALB/c mice																		
Nasal turb	5.8	5.6	6.3	6.4	6.3	6.0	3.7	3.2	3.3	5.3	5.1	5.1	5.3	5.0	5.0	2.4	—	2.6
Trachea	5.9	5.6	6.1	6.9	5.3	5.3	—	—	—	3.1	4.3	3.2	—	2.9	—	—	—	—
Lung	5.7	5.2	5.6	5.6	5.1	4.9	1.7	1.6	1.6	5.4	5.1	5.4	3.4	3.2	3.5	—	—	—
Hamsters (4 wk)																		
Nasal turb	NA[d]	NA	NA	6.7	6.5	6.5	5.2	—	2.5	NA	NA	NA	7.3	7.6	7.8	2.6	2.4	2.0
Trachea	NA	NA	NA	5.9	5.9	6.0	—	—	2.8	NA	NA	NA	3.4	5.6	5.7	—	—	—
Lung																		
R cra/acce	NA	NA	NA	6.5	6.8	—	—	—	2.8	NA	NA	NA	2.9	3.1	5.6	—	—	—
R middle	NA	NA	NA	6.8	6.6	—	—	—	3.2	NA	NA	NA	—	—	6.3	—	—	—
R caudal	NA	NA	NA	6.5	7.0	—	—	—	3.0	NA	NA	NA	2.7	2.7	6.2	—	—	—
L	NA	NA	NA	6.6	6.7	—	—	—	4.2	NA	NA	NA	—	5.4	6.5	—	—	—
Hamsters (8 wk)																		
Nasal turb	NA	NA	NA	6.7	6.9	6.1	4.3	3.0	3.3	—	NA	NA	6.7	7.9	6.9	3.0	2.6	3.1
Trachea	NA	NA	NA	5.6	6.2	5.7	—	2.2	—	—	NA	NA	4.2	6.0	6.1	—	—	—
Lung																		
R cra/acce	NA	NA	NA	6.6	3.3	2.8	4.4	—	—	NA	NA	NA	—	—	6.4	—	—	—
R middle	NA	NA	NA	6.8	3.1	2.6	4.8	—	—	NA	NA	NA	—	—	4.8	—	—	—
R caudal	NA	NA	NA	7.0	2.1	3.1	2.0	—	—	NA	NA	NA	2.9	—	6.6	—	—	—
L	NA	NA	NA	6.9	3.0	2.8	—	2.1	2.6	NA	NA	NA	—	1.7	3.7	—	—	—

[a]Six-week-old female DBA/2 mice and BALB/c mice and 4- or 8-week-old female Syrian hamsters were anesthetized and intranasally inoculated with 10^6 PFU of Victoria/2014 or Yamagata/2014 virus. Three animals per group were euthanized at 24 h postinfection (hpi) (mice only) and on days 3 and 6 postinfection.
[b]Nasal turb, nasal turbinate; R cra/acce, right cranial and accessory lobes; R middle, right middle lobe; R caudal, right caudal lobe; L, left lobe.
[c]—, virus not detected.
[d]NA, not available.

The distribution of sialic acids on the epithelial cells of the respiratory tract of ferrets (38) is similar to that on the epithelial cells of the respiratory tract of humans, in that SAα2,6Gal is dominant in the respiratory tract and SAα2,3Gal is expressed at low levels in the lower respiratory tract (39). In contrast, SAα2,3Gal is expressed in the respiratory tract of C57BL/6J mice, but SAα2,6Gal is not (40). These differences in receptor distribution might play a role in the differences in sensitivity to influenza viruses among animal species. Interestingly, the viruses tested in this study showed different cell tropisms. The type B viruses infected both olfactory epithelia and respiratory epithelia, but the H3N2 and pdmH1N1 viruses preferentially infected the olfactory epithelia (Fig. 5). The olfactory epithelial cells reacted with MAA II, which recognizes SAα2,3Gal, but not with SNA I, which recognizes SAα2,6Gal (Fig. 1B and D). Clinical human influenza viruses isolated in Madin-Darby canine kidney (MDCK) cells preferentially bind to SAα2,6Gal (1). Therefore, the distribution of these types of sialyloligosaccharides, as determined with MAA II and SNA I lectins, is not consistent with the receptor specificity of the viruses used. It may be that the SAα2,6Gal that is present in the hamster olfactory epithelia is not detectable with SNA I. Further studies are needed to test this possibility.

We also found that H1N1 and H3N2 viruses, but not type B viruses, are transmissible by the airborne route in hamsters (Fig. 6). Considering the difference in the virus titers in the nasal turbinates among these viruses, the transmissibility of influenza viruses in hamsters may depend on the virus titers in the upper respiratory tract. Although it is important to test whether the viruses can further transmit to other naive animals from exposed animals, we are currently unable to perform such an experiment due to the moratorium on gain-of-function experiments. Although the titers of type B virus in the

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TABLE 4 Number of antigen-positive cells in H3N2/2013-, pdmH1N1/2013-, and Victoria/2014-infected animals[a]

Animal and organ	No. of antigen-positive cells[b]											
	H3N2/2013				pdmH1N1/2013				Victoria/2014			
	Day 3		Day 6		Day 3		Day 6		Day 3		Day 6	
	1	2	1	2	1	2	1	2	1	2	1	2
DBA/2 mice												
Nasal turbinate	−	−	−	−	+	+	+	ND[c]	++	++	+	+
Trachea	−	−	−	−	+	+	+	ND	++	NA[d]	+	+
Bronchus	−	−	−	−	++	++	+	ND	++	++	+	++
Alveolus	−	−	−	−	++	++	++	ND	+	++	+	++
BALB/c mice												
Nasal turbinate	−	−	−	−	++	++	+	−	++	++	+	+/−
Trachea	−	−	−	−	+	+	+	+/−	++	++	+	+/−
Bronchus	−	−	−	−	++	+	+	+	++	++	+	+
Alveolus	−	−	−	−	++	+	+	+	++	+	+	+
Syrian hamster (4 wk)												
Nasal turbinate	−	+	−	−	+	+	+/−	+	++	++	+	+
Trachea	+	−	−	−	+	+	−	−	+	+	+/−	+/−
Bronchus	+	−	−	−	+	+	−	−	+	+	+/−	+/−
Alveolus	−	−	−	−	+/−	+/−	−	−	+/−	+/−	+/−	+/−

[a]Six-week-old female DBA/2 mice and BALB/c mice and 4-week-old female Syrian hamsters were anesthetized and intranasally inoculated with 10^6 PFU of H3N2/2013, pdmH1N1/2013, or Victoria/2014 virus. Two animals per group were euthanized on days 3 and 6 postinfection.

[b]−, no antigen-positive cells; +/−, less than 5 antigen-positive cells; +, more than 6 antigen-positive cells; ++, widespread antigen-positive cells.

[c]ND, not done. This animal died on day 3 postinfection.

[d]NA, not available.

nasal wash specimens were not particularly low, this virus was not transmissible by the airborne route (Fig. 6G). Nevertheless, our data suggest that hamsters may represent a useful model of transmission of influenza viruses. For evaluation of the airborne transmissibility of different influenza viruses, ferrets have been used extensively and guinea pigs have been used by some groups. It is important to compare these animal models side by side with the hamster model when evaluating the transmissibility of influenza viruses and its determinants.

In conclusion, hamsters have the potential to be a useful small-animal model for studies of influenza virus infection. They can be used for pathogenicity studies of not only the recent H3N2 viruses but also other strains. Moreover, they can also be used for studies of the transmission of some virus strains.

MATERIALS AND METHODS

Cells and viruses. MDCK cells were maintained in Eagle's minimal essential medium (MEM) containing 5% newborn calf serum at 37°C in 5% CO_2. For infectivity studies, we used three A(H3N2) viruses (A/Tokyo/IMS6-1/2013 [H3N2/2013], A/Tokyo/IMS2-1/2014 [H3N2/2014], and A/Tokyo/UT-HP002/2016 [H3N2/2016]), two A(H1N1)pdm09 viruses (A/Hiroshima/19/2013 [pdmH1N1/2013] and A/Tokyo/IMS1-1/2014 [pdmH1N1/2014]), and two type B viruses (a Victoria lineage virus, B/Kamakura/8/2014 [Victoria/2014], and a Yamagata lineage virus, B/Kamakura/10/2014 [Yamagata/2014]). For transmission studies, we used A/Texas/50/2012 (H3N2; TX50), A/Tokyo/IMS2-1/2014 (H3N2/2014), A/California/04/2009 (pdmH1N1; CA04), A/Brisbane/59/2007 (H1N1; BNE59), and B/Yokohama/UT-K1A/2011 (type B Victoria linage; UTK1A). All viruses except for BNE59 were isolated in MDCK cells or AX-4 cells (AX-4 cells are derivatives of MDCK cells expressing a larger amount of SAα2,6Gal) and then propagated them in MDCK cells. BNE59 was obtained from the U.S. CDC; it had been propagated in the allantoic cavity of embryonated chicken eggs.

Plaque assay. Viruses were diluted in MEM containing 0.3% bovine serum albumin (BSA). Confluent monolayers of MDCK cells were washed with MEM containing 0.3% BSA, infected with diluted viruses, and incubated for 30 to 60 min at 37°C. After the virus inoculum was removed, the cells were washed with MEM containing 0.3% BSA and overlaid with a 1:1 mixture of 2× MEM–0.6% BSA and 2% agarose containing 1 μg/ml tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-trypsin. The plates were incubated at 37°C for 48 h before virus plaques were counted.

Experimental infection. Six-week-old female BALB/c mice and DBA/2 mice and 4- or 8-week-old female Syrian hamsters (Japan SLC Inc., Shizuoka, Japan) were used for this study. The animal room was

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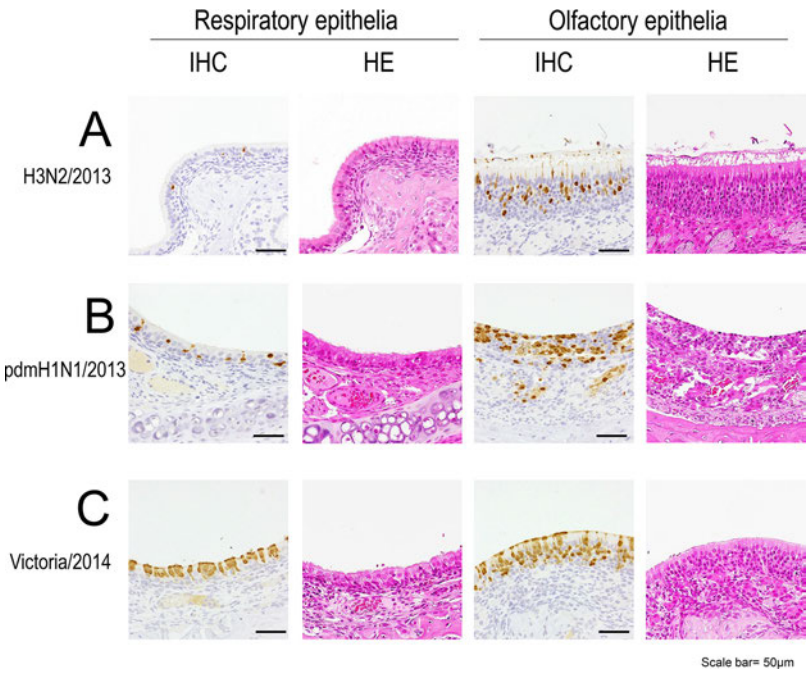

FIG 5 Pathological examination of the respiratory epithelia (left) and olfactory epithelia (right) of the nasal turbinates of infected 4-week-old hamsters. The images show the nasal turbinates of hamsters on day 3 postinfection with 10^6 PFU of H3N2/2013 (A), pdmH1N1/2013 (B), or Victoria/2014 (C) virus. HE, hematoxylin and eosin staining; IHC, immunohistochemistry for the detection of influenza virus NP antigen.

keep at 25°C and 50% humidity. Four mice and three hamsters per group were anesthetized with isoflurane and intranasally inoculated with 10^6 PFU/animal (50 μl for mice and 100 μl for hamsters) of H3N2/2013, H3N2/2014, H3N2/2016, pdmH1N1/2013, pdmH1N1/2014, Victoria/2014, or Yamagata/2014 viruses. Body weight and survival were monitored daily for 10 to 14 days postinfection (dpi). Baseline body weights were measured prior to infection. Nasal wash specimens were collected from each hamster with 400 μl of phosphate-buffered saline (PBS) every other day for virus titration. To assess virus growth in the respiratory organs, three mice or three hamsters per group were infected intranasally with 10^6 PFU of viruses and euthanized, and nasal turbinates, tracheas, and lungs were collected on days 1 (only for mice), 3, and 6 postinfection. The collected organs were homogenized with MEM containing 0.3% BSA and titrated in MDCK cells by using plaque assays.

Pathological examination. The excised respiratory tract tissues were fixed in 4% paraformaldehyde phosphate (PFA) buffer solution for 48 h and then processed for paraffin embedding. Nasal samples were immersed in EDTA solution for decalcification, after being fixed in PFA. The paraffin blocks were cut into 3-μm-thick sections and were mounted on silane-coated glass slides. To detect SAα2,6Gal and SAα2,3Gal, the sections were pretreated with 0.05% trypsin (Difco Laboratories, Detroit, MI, USA) at 37°C for 15 min and 0.3% hydrogen peroxide at room temperature for 30 min. They were then incubated at 4°C overnight with biotin-conjugated SNA I (EY Laboratories) for SAα2,6Gal detection and biotinylated conjugated MAA II (Vector Laboratories) for SAα2,3Gal detection. After being washed, the sections were then incubated with horseradish peroxidase-conjugated streptavidin and were visualized by staining with 3,3′-diaminobenzidine (DAB). The sections were also stained using a standard hematoxylin and eosin procedure, and each serial section was processed for immunohistological staining with a rabbit polyclonal antibody for type A influenza virus nucleoprotein and a mouse polyclonal antibody for type B influenza virus (prepared in the Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan). Specific antigen-antibody reactions were visualized with DAB staining by using a Dako Envision system (Dako Cytomation).

Airborne transmission study. For transmission studies in hamsters, animals were housed in transmission cages that had two wire-mesh partitions that prevented direct and indirect contact between animals but allowed the spread of influenza virus through the air (Showa Science) (Fig. 6A and B). Paper chips (Paper Clean; Japan SLC Inc.) were used for bedding to prevent the production of micropowder. The animal room was keep at 25°C and 50% humidity. Two 8-week-old hamsters were inoculated intranasally with 10^6 PFU (100 μl) of virus and placed in the larger room of the transmission cage (Fig. 6A) (day 0). At 24 h after infection (day 1), one naive 8-week-old hamster was placed in the smaller room adjacent to the inoculated hamsters. Three sets of hamsters (i.e., nine animals) were used for each virus tested. The hamsters were monitored for changes in body weight and the presence of clinical signs. To assess viral replication in nasal turbinates, we determined the virus titers in the nasal wash specimens collected from virus-inoculated and virus-exposed hamsters on day 2 after inoculation and then every other day.

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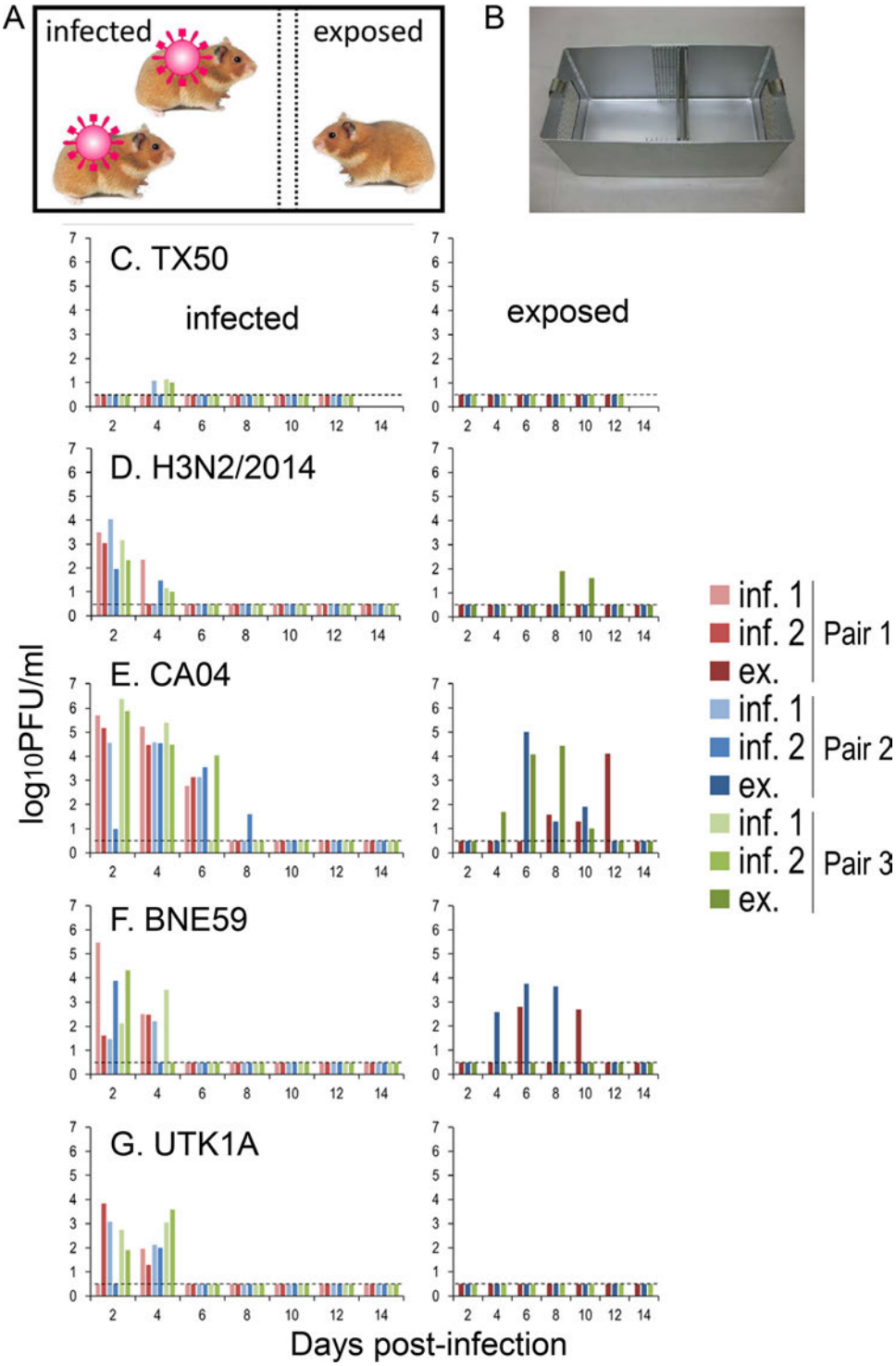

FIG 6 Respiratory droplet transmission of influenza viruses in hamsters. (A, B) Schematic representation (A) and photograph (B) of the transmission cage used for the hamster transmission studies. This cage has two wire-mesh partitions that prevent direct and indirect contact between the animals but allow the spread of influenza virus through the air. (C to G) Three groups of hamsters (two per group) were inoculated intranasally with 10^6 PFU of TX50 (C), H3N2/2014 (D), CA04 (E), BNE59 (F), or UTK1A (G) virus and then placed in the larger room of a transmission cage (day 0). At 24 h after infection (day 1), one naive exposed hamster per group was placed in the adjacent smaller room (A). Nasal washes were collected every other day from both infected (C to G, left) and exposed (C to G, right) animals for virus titration. Virus titers were determined by using a plaque assay on MDCK cells. The lower limit of detection is indicated by the horizontal dashed lines. inf., infected hamster; ex., exposed hamster.

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Ethics statements. Our research protocol for the animal studies is in accordance with the Regulations for Animal Care at the University of Tokyo, Tokyo, Japan, and was approved by the Animal Experiment Committee of the Institute of Medical Science, the University of Tokyo (approval numbers PA14-35 and PA15-10).

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CORRESPONDENCE

Transmission of SARS-CoV-2 in Domestic Cats

TO THE EDITOR: Reports of human-to-feline transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1] and of limited airborne transmission among cats[2] prompted us to evaluate nasal shedding of SARS-CoV-2 from inoculated cats and the subsequent transmission of the virus by direct contact between virus-inoculated cats and cats with no previous infection

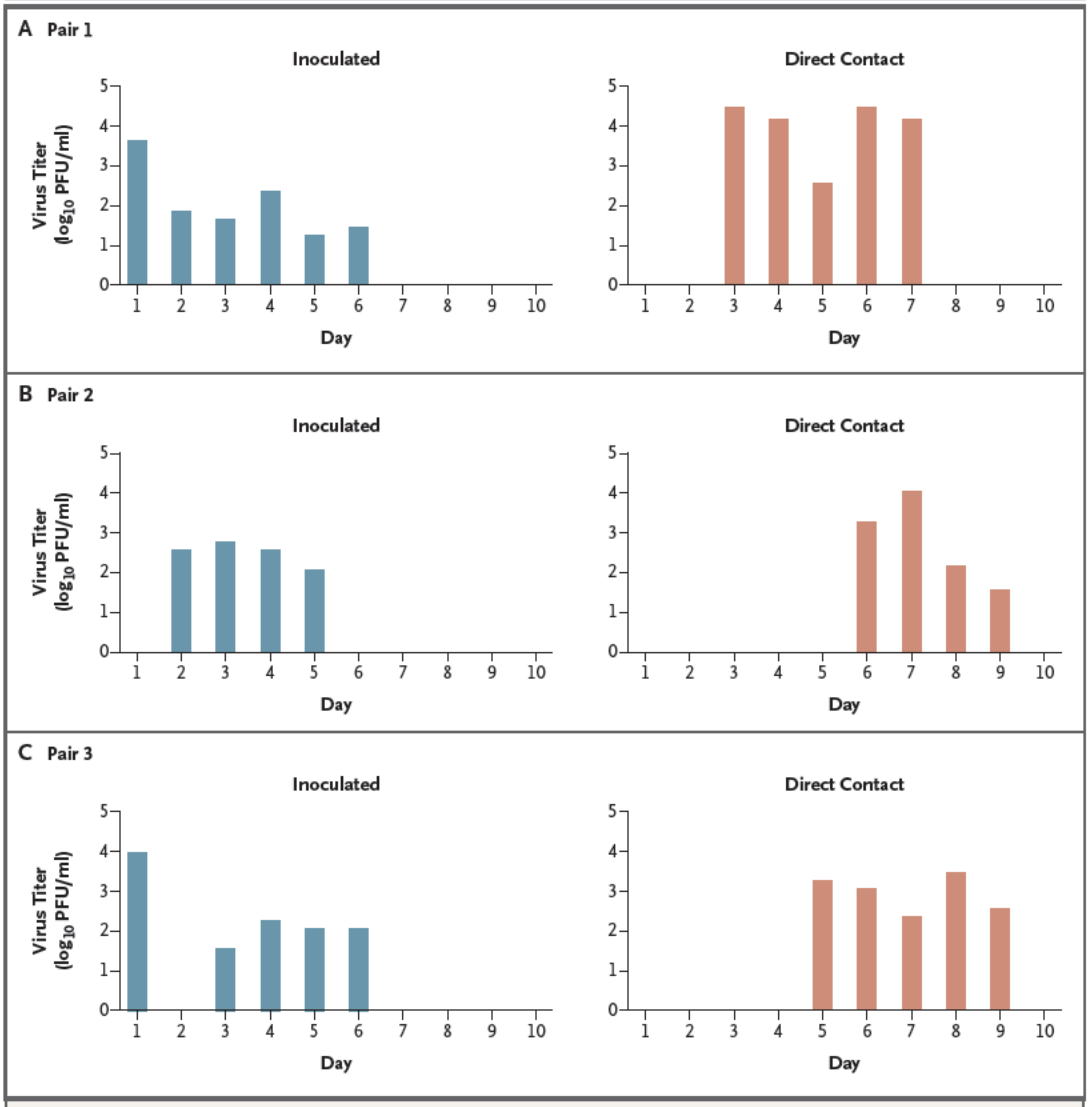

Figure 1. Virus Titers from Nasal Swab Specimens.
Three inoculated cats were infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on day 0. Three cats with no previous SARS-CoV-2 infection (direct contact) were cohoused in pairs (pairs 1, 2, and 3) with the inoculated cats on day 1. Nasal and rectal swab specimens were obtained on days 1 through 10. PFU denotes plaque-forming units.

with the virus. Three domestic cats were inoculated with SARS-CoV-2 on day 0. One day after inoculation, a cat with no previous SARS-CoV-2 infection was cohoused with each of the inoculated cats to assess whether transmission of the virus by direct contact would occur between the cats in each of the three pairs (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Nasal and rectal swab specimens were obtained daily and immediately assessed for infectious virus on VeroE6/TMPRSS2 cells.[3]

On day 1, we detected virus from two of the inoculated cats. By day 3, virus was detectable in all three inoculated cats, with continued detection of virus until day 5 in all cats and until day 6 in two of the three cats (Fig. 1).

The cats with no previous infection were cohoused with the inoculated cats on day 1. Two days later (day 3), one of the cats with no previous infection had infectious virus detected in a nasal swab specimen, and 5 days later, virus was detected in all three cats that were cohoused with the inoculated cats (Fig. 1). Virus titers in the cats that were cohoused with the inoculated cats peaked at 4.5 $\log_{10}$ plaque-forming units per milliliter, and virus shedding lasted 4 to 5 days (Fig. 1). No virus was detected in any of the rectal swabs tested. Although there have been reports of symptomatic infected cats, none of the cats in our study showed any symptoms, including abnormal body temperature, substantial weight loss (Fig. S1), or conjunctivitis. All the animals had IgG antibody titers between 1:5120 and 1:20,480 on day 24 after the initial inoculation.

With reports of transmission of SARS-CoV-2 from humans to domestic cats[1] and to tigers and lions at the Bronx Zoo,[4] coupled with our data showing the ease of transmission between domestic cats, there is a public health need to recognize and further investigate the potential chain of human–cat–human transmission. This is of particular importance given the potential for SARS-CoV-2 transmission between family members in households with cats while living under "shelter-in-place" orders. In 2016, an H7N2 influenza outbreak in New York City cat shelters[5] highlighted the public health implications of cat-to-human transmission to workers in animal shelters. Moreover, cats may be a silent intermediate host of SARS-CoV-2, because infected cats may not show any appreciable symptoms that might be recognized by their owners. The Centers for Disease Control and Prevention has issued guidelines for pet owners regarding SARS-CoV-2 (www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html). Given the need to stop the coronavirus disease 2019 pandemic through various mechanisms, including breaking transmission chains, a better understanding of the role cats may play in the transmission of SARS-CoV-2 to humans is needed.

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

This letter was published on May 13, 2020, at NEJM.org.

1. 2 Cats in NY become the first US pets to test positive for virus. AP News. April 22, 2020 (https://apnews.com/37328ab8db093b8346e26e1840b48af8).
2. Shi J, Wen Z, Zhong G, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science 2020 April 8 (Epub ahead of print).
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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Halfmann PJ, Hatta M, Chiba S, et al. Transmission of SARS-CoV-2 in domestic cats. N Engl J Med. DOI: 10.1056/NEJMc2013400

Table of Contents

Materials

Virus

The SARS-CoV-2 isolate, UT-NCGM02/Human/2020/Tokyo, was isolated in VeroE6 and was passaged twice on VeroE6 cells.

Cells

Vero E6/TMPRSS2 cells were obtained from the National Institute of Infectious Diseases, Japan. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and antibiotic/antimycotic (anti/anti) solution along with G418 (1 mg/ml).

Cats

The male and female domestic cats (15–18-week-old) used in this study were specific-pathogen-free animals from a research colony maintained at the University of Wisconsin-Madison. Animals were housed in 0.56 m x 0.81 m x 1.07 m cages in a laboratory with 65% humidity at 23ºC, and with at least 15.2 air exchanges per hour. Weight and body temperature (through implanted transponders) were measured daily (days 1–14). Blood (~0.5 ml) was collected in EDTA-tubes before infection (Day 0) and on Day 24.

Methods

Experimental Infection of Cats

Under ketamine and dexdomitor anesthesia, three cats were inoculated with 5.2 x 10^5 plaque-forming units (PFU) of SARS-CoV-2 given by a combination of inoculation routes for every animal (nasal [100 µl per nare], tracheal [500 µl], oral [500 µl], and ocular [50 µl per eye]). To reverse the effects of the anesthesia, antisedan was administered to the animals after completion of the inoculation.

Swab Sample Collection

Nasal and rectal swabs were collected daily during the study (Days 1–10). The swabs were soaked in DMEM prior to obtaining the nasal and rectal samples. After collection, the swabs were placed in a tube containing 1.0 ml of DMEM with anti/anti solution and vortexed for 1 minute in preparation for the virus titration assay.

Virus Titration Assay

Confluent Vero E6/TMPRSS2 cells in 12-well plates were infected with 100 µl of undiluted or 10-fold dilutions (10^{-1} to 10^{-5}) of the nasal or rectal swab sample. After a 30-minute incubation, the virus inoculum was removed, the cells were washed once, and then overlaid with 1% methylcellulose solution in DMEM with 5% FBS. The plates were incubated for three days, and then the cells were fixed and stained with 20% methanol and crystal violet in order to count the plaques.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA was performed using a recombinant receptor-binding domain (RBD) protein with a C-terminal HIS-tag purified by using TALON metal affinity resin from Expi293F cells (Thermo Fisher Scientific). The ELISA plates were coated overnight at 4 ºC with 50 µl of the RBD protein at a concentration of 2 µg/ml in phosphate-buffered saline (PBS). After blocking the plate with PBS containing 0.1% Tween 20 (PBS-T) and 3% milk powder, the plates with incubated in duplicate with heat-inactivated (56ºC for 30 minutes) serum diluted in PBS-T with 1% milk powder. After a four-hour incubation at room temperature, the plates were washed with PBS-T three times and then incubated with a cat IgG secondary antibody conjugated with horseradish peroxidase (Abcam; 1:10,000 dilution in PBS-T with 1% milk powder). After a one-hour incubation with the secondary antibody, the plates were washed three times with PBS-T and then developed with SigmaFast o-phenylenediamine dihydrochloride solution (Sigma). After a ten-minute incubation, the reaction was stopped with the addition of 3M hydrochloric acid. The absorbance was measured at a wavelength of 490 nm (OD_{490}). Background measurements from day 0 plasma was subtracted from the day 24 plasma for each dilution. IgG antibody titer was defined as the highest plasma dilution with an OD_{490} cut-off value of 0.15.

Biosafety Statement

The recombinant DNA protocol for the use of the virus was approved by the University of Wisconsin-Madison's Institutional Biosafety Committee. The cat transmission study with SARS-CoV-2 was performed in biosafety level 3 agriculture (BSL-3Ag) laboratories at the Influenza Research Institute. The laboratory is approved for such use by the Centers for Disease Control and Prevention. The BSL-3Ag facility used was designed to exceed the standards outlined in *Biosafety in Microbiological and Biomedical Laboratories* (5th edition).

Features of the BSL-3Ag facility include controlled access, entry/exit through a shower change room, effluent decontamination, negative air-pressure, double-door autoclaves, gas decontamination ports, HEPA-filtered supply and double-HEPA-filtered exhaust air, double-gasketed watertight and airtight seals, and airtight dampers on all ductwork. The structure of the BSL-3Ag facility is pressure-decay tested regularly.

Supplemental Tables and Figures

Table 1. Age and sex of the cats used in the study.

Pairs		Age at time of infection (day 0)	Sex
Pair 1	Infected	15 weeks	Female
	Contact	15 weeks	Female
Pair 2	Infected	18 weeks	Male
	Contact	18 weeks	Male
Pair 3	Infected	15 weeks	Female
	Contact	15 weeks	Female

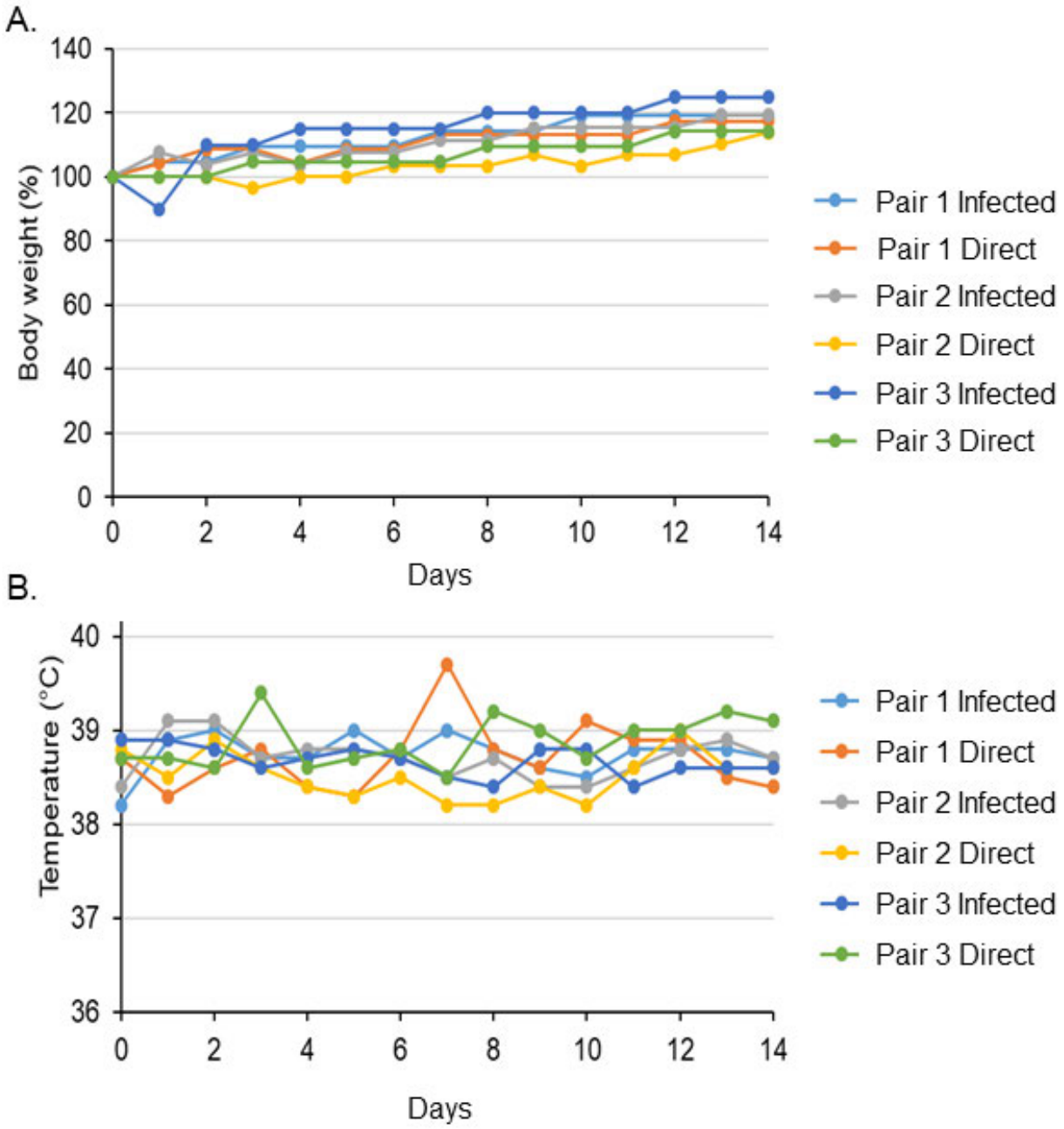

Figure 1. Changes in body weight (A) and body temperature (B) of SARS-CoV-2-infected and direct contact cats in each of the three groups studied.

Acknowledgments

We would like to thank Tammy Armbrust, Aaron Balogh, Chunyang Gu, Lizheng Guan, Huihui Kong, Erin Plisch and Hongyu Rao for experimental assistance, Dr. Florian Krammer for the protein expression vector for the soluble SARS-CoV-2 receptor-binding domain, and Sue Watson for scientific editing.

This research was supported by the National Institutes of Allergy and Infectious Diseases funded Center for Research on Influenza Pathogenesis (CRIP; HHSN272201400008C to Dr. Kawaoka) and by a Research Program on Emerging and Re-emerging Infectious Disease from AMED (19fk0108113 to Dr. Kawaoka).

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: 229E, OC43, HKU1
Date: Friday, March 27, 2020 9:15:00 PM

[redacted]

We are seeking human coronaviruses to use as comparison viruses for CoV-2. Would you be willing to share stocks of 229E, OC43, and/or HKU1 with us if you have them? Or can you suggest an alternative PI to contact for these viruses?

Best,
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: [redacted]work, contract activities during shutdown
Date: Monday, March 30, 2020 5:36:00 AM

Dear All,

Work on the [redacted] will continue since we are currently at a stage where large datasets need to be analyzed (for example, the sequences of variants isolated from [redacted] need to be analyzed; recently obtained HI and FRA data need to be analyzed [redacted] have to be generated and analyzed). In addition, we are currently working on manuscripts.

Yours,

[redacted]

-----Original Message-----
From: [redacted]
Sent: Friday, March 27, 2020 5:09 AM
To: [redacted]
Cc: [redacted]
Subject: [redacted]work, contract activities during shutdown

Hi all,

For [redacted] questions re contract activities during the shutdown, [redacted] would like us please to coordinate on our replies to [redacted] for our [redacted] options. This will allow us to better coordinate with [redacted] re [redacted] activities. Let's discuss in our call today if we have time. The questions are below FYI:

- Are you working in the lab on flu right now?

- If you are not working on flu what [redacted] related activities are you doing at remotely (examples writing papers, data analysis, lab meetings)?

- Are you working on COVID using [redacted] funds? If yes have you cleared this with [redacted]? How will this impact your flu studies?

Many thanks and hope you are all well,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] for the [redacted] meeting
Date: Saturday, March 28, 2020 4:42:29 PM

Hi [redacted] and All,

I looked at the data again; here are two high-level observations:

- [redacted] The [redacted] are small, but you may remember that they were also small with the [redacted] mutants. With the [redacted] mutants, greater [redacted] changes were detected for the [redacted]' mutants.
- Across the viruses tested ([redacted]), most of the [redacted] mutants seem to possess changes at position [redacted] (I did not perform statistical analyses … I just moused-over the mutants …). Is this something we should think about more (Additional testing? Publication?) – just some food for thought.

Thanks,

[redacted]

From: [redacted]
Sent: Friday, March 27, 2020 5:55 AM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] for the [redacted] meeting

Hi everyone,

Please see links to two webpages we'll be discussing in the call. If you have any trouble accessing them please let me know.

[redacted]

Best,
[redacted]

On 26 Mar 2020, at 09:53, [redacted] wrote:

Hi [redacted]

Yes we will present current [redacted] status. Also, to communicate our plans re the [redacted] map, we will give a brief overview of the investigations we are deep into, and that I wrote about, re judging if [redacted] we will keep that high-level and leave detail and discussion for when you have more time.

We'd also like to discuss the [redacted] rough vaccine sera I also wrote about, and how this will be very helpful, likely essential, for coming to a CVV decision.

[redacted]

On Wed, Mar 25, 2020 at 1:11 PM [redacted] wrote:

> [redacted], thanks, I received it.
>
> [redacted] and [redacted] earlier emails suggested that you have updates on the [redacted] perhaps as well?). Could you present your updates on Friday?
>
> Thanks a lot,

From:
Sent: Wednesday, March 25, 2020 6:56 AM
To:
Cc:
Subject: Re: for the meeting

Dear all,

You should now have received a webex invite for this meeting on Friday 27th at 6 am CT / 7 am ET / 11 am UK / 12 pm NL / 8 pm Japan.

If it has not come through for any reason please let me know.

Thanks

On 25 Mar 2020, at 09:37, wrote:

Should also be fine for me

From:
Date: Wednesday, 25 March 2020 at 08:45
To:
Cc:
Subject: Re: for the meeting

Yes, that would work for me.

Yours sincerely,

CC:

[redacted]: FW: [redacted] for the [redacted] meeting

Dear All,

Are we (still) planning to have a call on Friday (3/27) to discuss the latest [redacted] data (as needed), and recent updates on the [redacted] in preparation for the [redacted] meeting in April?
A possible time would be 6 am CT / 7 am ET / 11 am UK / 12 pm NL / 8 pm Japan.
[redacted] and [redacted] you were not cc'd on the previous email – linking you in now.

Thanks,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]

Subject: RE: [redacted] work, contract activities during shutdown
Date: Sunday, March 29, 2020 7:46:00 AM

[redacted]

Thank you for sharing the information!

[redacted]

-----Original Message-----
From: [redacted]
Sent: Sunday, March 29, 2020 9:39 PM
To: [redacted] >
Cc: [redacted]
Subject: Re: [redacted] work, contract activities during shutdown

Hi [redacted]
I already responded about my [redacted] contract (see below) because I think that [redacted] would coordinate a response for the [redacted] only, and it would be unlogical if he would also write about our [redacted] contracts and options on completely different topics.
Kind regards
[redacted]

[redacted]
Our labs have completely shut down for non-essential work, but we continue essential work including work on coronavirus. In practice, this means that our [redacted] work is still somewhat continuing, in particular because of the ongoing detections of [redacted] Our PhD students, post-docs, PIs are mostly working on data analyses and manuscript writing. In particular the folks that are on the Options.

Some of the technical personnel and animal experimentalists have been partly shifted to assist in the diagnostics (e.g. setting up new methods now that there is a shortage on diagnostic reagents), NGS (implementing real-time minion NGS, similar to what we developed with [redacted]), to macaque experiments to study pathogenesis, to study virus transmission in ferrets and to measure virus in aerosols and droplets. This is all done with personnel on the base contract.

Kind regards,

Yours sincerely,
[redacted]

Yours sincerely,

[redacted]

How are you going to respond to the request, considering that cannot be re-directed? As suggested, we may want to coordinate our response.

Best,

-----Original Message-----
From:
Sent: Friday, March 27, 2020 5:09 AM
To:
Cc:
Subject: work, contract activities during shutdown

Hi all,

For questions re contract activities during the shutdown, would like us please to coordinate on our replies to for our This will allow us to better coordinate with re activities. Let's discuss in our call today if we have time. The questions are below FYI:

- Are you working in the lab on flu right now?

- If you are not working on flu what related activities are you doing at remotely (examples writing papers, data analysis, lab meetings)?

- Are you working on COVID using funds? If yes have you cleared this with ? How will this impact your flu studies?

Many thanks and hope you are all well,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Cancellation FW: Invitation: Influenza and other infecition, June 16-17, 2020
Date: Saturday, March 21, 2020 10:36:21 AM

Dear [redacted], thanks for the update. I'd be delighted to participate in the future. Stay safe! [redacted]

From: [redacted]
Sent: Friday, March 20, 2020 8:52 PM
To: [redacted]
Cc: [redacted]
Subject: Cancellation FW: Invitation: Influenza and other infecition, June 16-17, 2020

Dear [redacted]

I am writing to inform you that we have decided to cancel our symposium due to the COVID-19 outbreak.

Although we will not reschedule this symposium, I plan to organize another symposium when the COVID-19 outbreak calms down. I hope that you will consider coming to that symposium, although I do not yet know when we will be able to have such an event.

Best,

[redacted]

[redacted]

From: [redacted]
Sent: Sunday, October 27, 2019 9:18 AM

To: [redacted]
Cc: [redacted]
Subject: RE: Invitation: Influenza and other infecition, June 16-17, 2020

Dear [redacted]

Great! Thank you for accepting my invitation.
The secretariat will provide you with the logistical information in due course.

The website was for last year's meeting. For next year, I have invited you, [redacted]. So far, [redacted], and you have agreed to participate. [redacted] is checking his schedule to make sure it doesn't overlap with a WHO meeting.

If you could give me a general title in a couple of weeks, that would be great. But, the secretariat will contact you to request a tentative title, a short bio, and a headshot.

Best,
[redacted]

From: [redacted]
Sent: Sunday, October 27, 2019 7:55 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Invitation: Influenza and other infecition, June 16-17, 2020

Hi [redacted] Nice to hear from you. I will be delighted to attend. It looks like a great program and should be very interesting. Hope things are going well with you. How soon do you need a title? [redacted]

From: [redacted]
Sent: Friday, October 25, 2019 8:03 AM
To: [redacted]
Cc: [redacted]
Subject: Invitation: Influenza and other infecition, June 16-17, 2020

Dear [redacted],

I am writing to see if you could give a talk on your work at a symposium I am organizing in Tokyo, Japan on June 16-17, 2020. The title of the symposium is

“Influenza and other infections”. This year’s website for this symposium is:

https://www2.aeplan.co.jp/sioi/index.html

With regard to support for your travel, I am providing 500,000 yen ($4,546 at a rate of 1 dollar=110 yen), with which I am asking you to cover your airfare, local transportation, and per diem. Of course, we will cover your hotel for the evenings of June 15-17th, 2020 and meals during the symposium.
I hope that you are interested in coming to Tokyo and sharing your interesting research.
I look forward to your positive response.

Best,
[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Covid-19 impact on [redacted]
Date: Monday, March 16, 2020 7:03:29 AM

[redacted]

Thanks. We will hold the shipment for now.

We now have cases here in Madison and the surrounding areas, including one at the [redacted] Since we are in a separate building a few miles away, we remain operational for now.

Cheers,

[redacted]

-----Original Message-----
From: [redacted]
Sent: Monday, March 16, 2020 4:21 AM
To: [redacted]>
Cc: [redacted]>
Subject: Re: Covid-19 impact on [redacted]

Dear [redacted]
Thanks a lot. We will keep you updated with our situation.
Best
[redacted]

On 13/03/2020, 23:37, [redacted]> wrote:

[redacted],

We were planning to ship viruses and sera on Monday, but will hold the shipment until we get word that you are ready to receive it.

All the best,

[redacted]

-----Original Message-----
From: [redacted]
Sent: Friday, March 13, 2020 4:48 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Covid-19 impact on [redacted]

[redacted]
Thank you for letting us know.
[redacted]

-----Original Message-----
From: [redacted]
Sent: Saturday, March 14, 2020 4:33 AM
To: [redacted]
Cc: [redacted]
Subject: Covid-19 impact on [redacted]

Dear [redacted]

Please be aware that we have received an update from [redacted], with the exception of except for SARS-CoV2 work and very essential/urgent lab work.

This will have consequences on the work that they do under the [redacted] contract.

Thanks

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Invitation to the 7th ESWI Influenza Conference Programme Committee TC
Date: Monday, March 16, 2020 5:38:00 AM

Dear [redacted]

I will not be able to join.
It will be 1:00 am in Tokyo.

[redacted]

-----Original Message-----
From: [redacted] >
Sent: Monday, March 16, 2020 6:51 PM
To: [redacted]
Cc: [redacted]
Subject: Invitation to the 7th ESWI Influenza Conference Programme Committee TC

Dear all

I hope you are all well. I'm contacting you on behalf of [redacted], chairs of the 7th ESWI Influenza Conference to invite you to the first TC of the programme committee.

The TC is scheduled on Friday March 20, 2020 at 17.00 CET (Brussels time). We will send you a calendar invite, agenda and meeting documents shortly.

Best regards
[redacted]

Please join my meeting from your computer, tablet or smartphone.
[redacted]

You can also dial in using your phone.
Access Code: [redacted]

phone numbers
[redacted]

New to GoToMeeting? Get the app now and be ready when your first meeting starts:

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison
Date: Thursday, March 5, 2020 2:19:00 PM

[redacted]

Please let me know your postdoc's email address.
We need the detail of the construct to get approval to use your recombinant viruses; this will be reviewed by the National Committee for rDNA experiments, which usually takes 6 months to issue an approval.

[redacted] (CCed here) is in charge of the rDNA paperwork.

Best,
[redacted]

From: [redacted]
Sent: Thursday, March 5, 2020 1:18 PM
To: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

We have [redacted] Final stocks are being titered. I'll talk with the postdoc who made the virus. Glad to hear your [redacted]

From: [redacted]
Sent: Wednesday, March 4, 2020 8:42 AM
To: [redacted] >
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Dear [redacted],

I understand. Thank you for your kind consideration!

Regarding the submission of the [redacted] grant, I will follow your recommendation.

For our in vivo imaging work, we are now using a variety of reporter mice (see

attached; neutrophils and macrophages are color-labeled). When you have recombinant SARS-CoV-2 expressing a fluorescent protein, please let me know. We can do some imaging studies with your viruses.

Best,

[redacted]

P.S. I now understand why we got a good score for our [redacted]!

From: [redacted]
Sent: Wednesday, March 4, 2020 9:52 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

[redacted] currently we have 18 breeders with about 8 still aging up to ~5 weeks before they can enter our breeding funnels. If we send breeder pairs to everyone who is asking now, then I'm cutting my own research throat. We anticipate having a lot more mice in early april at which time, we can begin to send out breeding sets to group. We are also looking to freeze down sperm from males. At this time, don't expect a ton of mice. I haven't been able to do a single experiment myself with these animals, as we were just about to cryopreserve the entire line, and then the novel cov emerged. All efforts have been on expanding our colony.

We haven't been able to do the [redacted] experiment yet, although we have animals ready. [redacted] is scheduled to do these experiments the 2nd week of april, which puts me in a difficult position regarding the [redacted] grant. Having just sat in on [redacted] my feeling is that our chances of funding are minimal without the [redacted] data. I'm thinking we should delay to july 5th, although I would like to have it submitted prior to that date.

[redacted]

From: [redacted]
Sent: Tuesday, March 3, 2020 11:02 AM
To: [redacted]
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Dear [redacted]

>I don't think I was included on the first few emails

The following is the initial email exchange I had with [redacted].

>could you let me know what your animal request was

If [redacted] could provide us with a couple hACE2 males, that would be great.

Best,
[redacted]

From: [redacted]
Sent: Thursday, January 30, 2020 11:41 AM
To: [redacted]
Cc: [redacted]
Subject: RE: hACE2 transgenic mice

Hi [redacted], We are going to need to set up a MTA agreement with Univ Wis. Madison for the transgenic hACE2 mice that are available in my laboratory. I will send you a blerb describing the mice shortly. [redacted] I will need contact information for the people who deal with these things at UW-Madison. Talk with you soon. [redacted]

From: [redacted]
Sent: Wednesday, January 29, 2020 12:22 PM
To: [redacted]
Cc: [redacted] >
Subject: hACE2 transgenic mice

Dear [redacted],

As I mentioned, I will be testing the growth of this virus in animals including marmosets, cats, dogs, ferrets, hamsters, and mice. I will also be examining vaccine candidates.

To this end, I am interested in obtaining your hACE2 transgenic mice. If you are going to examine the replication of 2019-nCoV in hACE2 transgenic mice and perform pathological analyses etc., we will not perform such studies.

Please let me know how we should proceed.
Thank you for your help!

[redacted]

From: [redacted]
Sent: Tuesday, March 3, 2020 11:43 PM
To: [redacted] >
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Hi [redacted]

Thank you so much for the update! We did not have anything specific set-up just an overall request. I have cc'd [redacted] and [redacted] who can advise what we would like to receive. We greatly appreciate your willingness to keep us updated.

[redacted] – Could you let [redacted] know what you would like to receive?

Thanks!
[redacted]

From: [redacted] >
Sent: Tuesday, March 3, 2020 8:32 AM
To: [redacted] >
Subject: Re: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Hey [redacted],

It's hard to give an estimated date right now since we are having to grow up our colony first. I am weaning the first few litters this week (most are first time breeders), but we still have breeders we need to set up from those litters. I would guess that we won't have any animals to send before April/beginning of May?

I don't think I was included on the first few emails, could you let me know what your animal request was and I can try and keep you updated when we start increasing our colony and getting closer to being able to send off any animals.

[redacted]

From: [redacted]
Sent: Monday, March 2, 2020 2:31 PM
To: [redacted] >
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Hi [redacted]

Do you have an estimated date of when animals will be available?

Thank you!
[redacted]

From: [redacted]
Sent: Friday, February 21, 2020 4:26 PM
To: [redacted]
Cc: [redacted]
[redacted] >
Subject: Re: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

I've passed this request on to our animal technician, [redacted]. When we have mice available she will be able to set up the transfer.

[redacted]

> On Feb 20, 2020, at 5:04 PM, [redacted] > wrote:
>
> Good Afternoon [redacted]
>
> [redacted], will need to review the colony health report before approval to ship the mice. Please let us know the strain, quantity, sex and age of the mice. I have an account with World Courier and Validated Delivery to cover the shipping cost.
>
> Best Regards
> [redacted]
>
> [redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison
Date: Wednesday, March 4, 2020 7:42:00 AM
Attachments: Ueki Nature Protocol.pdf

Dear [redacted]

I understand. Thank you for your kind consideration!

Regarding the submission of the [redacted] grant, I will follow your recommendation.

For our in vivo imaging work, we are now using a variety of reporter mice (see attached; neutrophils and macrophages are color-labeled). When you have recombinant SARS-CoV-2 expressing a fluorescent protein, please let me know. We can do some imaging studies with your viruses.

Best,

[redacted]

P.S. I now understand why we got a good score for our [redacted]!

From: [redacted]
Sent: Wednesday, March 4, 2020 9:52 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

[redacted], currently we have 18 breeders with about 8 still aging up to ~5 weeks before they can enter our breeding funnels. If we send breeder pairs to everyone who is asking now, then I'm cutting my own research throat. We anticipate having a lot more mice in early april at which time, we can begin to send out breeding sets to group. We are also looking to freeze down sperm from males. At this time, don't expect a ton of mice. I haven't been able to do a single experiment myself with these animals, as we were just about to cryopreserve the entire line, and then the novel cov emerged. All efforts have been on expanding our colony.

We haven't been able to do the [redacted] experiment yet, although we have animals ready. [redacted] is scheduled to do these experiments the 2nd week of april, which puts me in a difficult position regarding the [redacted] grant. Having just sat in on [redacted], my feeling is that our chances of funding are minimal without the [redacted] data. I'm thinking we should delay to july 5th, although I would like to have it submitted prior to that date.

[redacted]

From: [redacted]
Sent: Tuesday, March 3, 2020 11:02 AM
To: [redacted]
Cc: [redacted]
[redacted]>
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Dear [redacted]

>I don't think I was included on the first few emails

The following is the initial email exchange I had with [redacted]

>could you let me know what your animal request was

If [redacted] could provide us with a couple hACE2 males, that would be great.

Best,
[redacted]

From: [redacted]
Sent: Thursday, January 30, 2020 11:41 AM
To: [redacted]
[redacted]>
Cc: [redacted]
Subject: RE: hACE2 transgenic mice

Hi [redacted], We are going to need to set up a MTA agreement with Univ Wis. Madison for the transgenic hACE2 mice that are available in my laboratory. I will send you a blerb describing the mice shortly. [redacted] I will need contact information for the people who deal with these things at UW-Madison. Talk with you soon. [redacted]

From: [redacted]
Sent: Wednesday, January 29, 2020 12:22 PM
To: [redacted]

Cc: [redacted]
Subject: hACE2 transgenic mice

Dear [redacted]

As I mentioned, I will be testing the growth of this virus in animals including marmosets, cats, dogs, ferrets, hamsters, and mice. I will also be examining vaccine candidates.

To this end, I am interested in obtaining your hACE2 transgenic mice. If you are going to examine the replication of 2019-nCoV in hACE2 transgenic mice and perform pathological analyses etc., we will not perform such studies.

Please let me know how we should proceed.
Thank you for your help!

[redacted]

From: [redacted]
Sent: Tuesday, March 3, 2020 11:43 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Hi [redacted]

Thank you so much for the update! We did not have anything specific set-up just an overall request. I have cc'd [redacted] and [redacted] who can advise what we would like to receive. We greatly appreciate your willingness to keep us updated.

[redacted] – Could you let [redacted] know what you would like to receive?

Thanks!
[redacted]

From: [redacted]
Sent: Tuesday, March 3, 2020 8:32 AM
To: [redacted]
Subject: Re: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Hey [redacted],

It's hard to give an estimated date right now since we are having to grow up our colony first. I am weaning the first few litters this week (most are first time breeders), but we still have breeders we need to set up from those litters. I would guess that we won't have any animals to send before April/beginning of May?

I don't think I was included on the first few emails, could you let me know what your animal request was and I can try and keep you updated when we start increasing our colony and getting closer to being able to send off any animals.

[redacted]

From: [redacted]
Sent: Monday, March 2, 2020 2:31 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

Hi [redacted],

Do you have an estimated date of when animals will be available?

Thank you!
[redacted]

From: [redacted]
Sent: Friday, February 21, 2020 4:26 PM
To: [redacted]
Cc: [redacted]
[redacted]
[redacted]>
Subject: Re: Mouse Transfer [redacted] at UNC to [redacted] at UW-Madison

I’ve passed this request on to our animal technician, [redacted] When we have mice available she will be able to set up the transfer.

[redacted]

> On Feb 20, 2020, at 5:04 PM, [redacted]>
> wrote:

Good Afternoon [redacted]

[redacted], will need to review the colony health report before approval to ship the mice. Please let us know the strain, quantity, sex and age of the mice. I have an account with World Courier and Validated Delivery to cover the shipping cost.

Best Regards

[redacted]

[redacted]

nature protocols

PROTOCOL

https://doi.org/10.1038/s41596-019-0275-y

Multicolor two-photon imaging of in vivo cellular pathophysiology upon influenza virus infection using the two-photon IMPRESS

Hiroshi Ueki[1], I-Hsuan Wang[1], Dongming Zhao[1,2], Matthias Gunzer[3] and Yoshihiro Kawaoka[1,4,5*]

In vivo two-photon imaging is a valuable technique for studies of viral pathogenesis and host responses to infection in vivo. In this protocol, we describe a methodology for analyzing influenza virus-infected lung in vivo by two-photon imaging microscopy. We describe the surgical procedure, how to stabilize the lung, and an approach to analyzing the data. Further, we provide a database of fluorescent dyes, antibodies, and reporter mouse lines that can be used in combination with a reporter influenza virus (Color-flu) for multicolor analysis. Setup of this model typically takes ~30 min and enables the observation of influenza virus-infected lungs for >4 h during the acute phase of the inflammation and at least 1 h in the lethal phase. This imaging system, which we termed two-photon IMPRESS (imaging pathophysiology research system), is broadly applicable to analyses of other respiratory pathogens and reveals disease progression at the cellular level in vivo.

Introduction

In vivo two-photon imaging is an analytical approach that can be used to visualize cell dynamics and hemodynamics in organs or tissues of live animals. Information in real time obtained by using this approach, such as changes in cell behavior and morphology, tissue localization, and blood flow, has revealed highly sophisticated and dynamic systems of living organisms. During in vivo imaging, the blood circulation in the tissue being observed is maintained; therefore, this technique is also effective for analyzing the migration and invasion of immune cells in the inflammatory environment. Observations in physiological environments deepen our understanding of host response mechanisms under both steady-state and disease conditions.

Computed tomography, X-ray, and IVIS Spectrum (an in vivo imaging system) imaging methods have been used as non-invasive approaches; however, these techniques have low spatiotemporal resolution and have been able to estimate only the site of inflammation in an organ[1,2]. Therefore, it is impossible to observe cellular responses of the immune system using these approaches. By contrast, a two-photon excitation laser microscope, the light source of which is a near-infrared laser that produces low damage to cells but has long-reaching depth in tissue, enables us to capture the movement of cells in living animals at high resolution. Two-photon imaging has been in use in biological science since the 1990s; it has progressed at a remarkable rate, and observation methods for various organs, including brain, liver, and lymph nodes, have been reported[3,4]. In this protocol, we describe how to use it to image virus-infected lungs. We have previously demonstrated that this protocol works by using mice infected with mouse-adapted seasonal influenza virus (H1N1) or highly pathogenic avian influenza virus (H5N1)[5].

Challenges when imaging the lung

The lung, which is a respiratory organ, has contact with the outside environment and is an important organ for research on immunity to infectious diseases. In the seventeenth century, Marcello Malpighi

[1]Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan. [2]State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, People's Republic of China. [3]Institute for Experimental Immunology and Imaging, University Hospital, University Duisburg-Essen, Essen, Germany. [4]Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan. [5]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin, USA. *e-mail: yoshihiro.kawaoka@wisc.edu

discovered pulmonary capillaries and alveoli in the frog lung by using optical microscopy[6]; now fluorescent reporter mice facilitate the study of disease models in conjunction with two-photon excitation microscopy (Table 1). However, a challenge encountered when imaging the lung is that it is constantly moving during respiration. The lung has been stabilized in several ways during in vivo observation by microscopy, including bronchus clamping, prolonged apnea, gluing, and suction[7,8]; however, it is difficult to reduce motion artifacts due to lung respiratory movement under physiological conditions and hence to obtain high-quality images. Bronchus clamping can suppress respiratory motion artifacts of the lung lobe[9,10]; however, it is not suitable for long-term observation because it causes severe hypoxia. Although prolonging apnea is less invasive[11–13], it does not allow researchers sufficient time to observe the lung for image acquisition by two-photon excitation microscopy, and the quality of the images tends to deteriorate over time. Gluing addresses the above limitations[14,15]; however, it can induce shear force injury and inflammation, which affect the interpretation of results. A suction window, which is currently the most commonly used stabilizing system during lung imaging, achieves moderate immobilization of the lung and high-quality images[16–19]; however, the observation period is limited to ≤12 h. Ex vivo imaging of lungs and in vivo imaging of trachea have also been performed as complementary methods[8]. Each of these methods has its advantages and disadvantages, and it is important to select and optimize the method best suited to the goal of the experiments and disease model.

In vivo observation of lungs has been performed using various lung disease and experimental models, including bacterial infection, allergen inoculation, tumor metastasis, and lipopolysaccharide (LPS)-induced sepsis (Table 1). However, for viral respiratory diseases, such as influenza, other than an observation in a methodology report[20], only analyses of the trachea in vivo[21–23] and isolated lungs had been performed[24], with no analysis of the lung in vivo, until our recent publication[5] (Table 1). Unlike ex vivo methods, which involve isolated or sliced lungs, in vivo imaging using two-photon excitation microscopy of live animals enables researchers to observe hemodynamics, migration and extravasation of immune cells, as well as interactions among immune cells during influenza virus infection. However, it is technically demanding to perform two-photon excitation microscopy of live influenza virus–infected lung, which exhibits severe inflammation, requiring the development of highly sophisticated, less invasive instruments and surgical techniques. In addition, when observing animals infected with pathogenic viruses, specialized facilities and instruments are frequently required to avoid the spread of the virus. Furthermore, because many types of immune cells infiltrate the infected lung in an inflammatory environment, it is necessary to distinguish the target immune cells from the infected cells by using fluorescent labels in the infected microenvironment. To detect multiple fluorescent signals excited simultaneously by a two-photon excitation laser, fluorochromes with different spectra and equal brightness must be selected; however, there is currently no comprehensive database of fluorescent reagents, fluorescent reporter viruses, and reporter mouse lines available for lung in vivo imaging. We therefore also provide a database of fluorescent dyes, antibodies, and reporter mouse lines that can be used in combination with a reporter influenza virus (Color-flu)[25–27] for multicolor analysis under pathological conditions in this protocol.

Our system uses suction-based lung stabilization[16,28] to improve an existing in vivo two-photon imaging system for influenza virus–infected lung as a model of an acute inflammatory respiratory disease[5]. We have successfully used C57BL/6 mice and transgenic mice of the C57BL/6 background (6- to 10-week-old males and females). By using our method, described in detail here, it is possible to visualize and analyze the behavior of immune cells and their interactions with infected cells during an influenza virus infection, which creates an acute inflammatory environment.

Limitations of the protocol

A limitation of two-photon excitation microscopy is that the observation depth that can be achieved is a maximum of ~70 μm. Therefore, we cannot observe the bronchial region. This limitation is linked to the wavelength of the infrared laser and detector capability of the microscope. However, as laser technology develops, the observation depth achievable using this method will improve.

Applications of the protocol

In this protocol, we describe the application of this methodology to influenza virus infection of the lungs because this is what we have used it for previously. This protocol could be applied not only to studies of the early stages of inflammation due to infection or other causes, but also to analyses of tissue regeneration mechanisms in lungs that are in the process of recovering from infection or other

Table 1 | Summary of the disease and experimental models used for in vivo microscopic observation of the lung

Disease/experimental model	Technique	Animal model	Year	Ref.	Disease/experimental model	Technique	Animal model	Year	Ref.
Steady state	Clamping	Cats, rabbits	1925	48	Hypoxia	Suction	Dogs	1975	49
	Window approach	Cats	1926	50		Suction	Dogs	1979	51
	Manual tracking	Dogs, frogs, alligators	1930	52		Suction	Dogs	1981	53
	Clamping	Rabbits, cats, dogs	1933	9		Suction	Dogs	1982	54
	Window approach/curare	Cats	1934	55		Suction	Rabbits	1992	56
	Suction	Cats	1939	57		Prolonged apnea	Mice	2008	12
	Window approach	Dogs	1965	58		Prolonged apnea	Mice	2013	59
	Suction	Dogs	1969	60	Ischemia-reperfusion injury	Prolonged apnea	Rats	1999	11
	Suction	Dogs	1982	61		Prolonged apnea	Rats	1999	62
	Suction	Dogs	1987	63		Glue	Mice	2010	14
	Window approach/ pancuronium	Rabbits	1989	64		Glue	Mice	2011	65
	Suction	Dogs	1992	66		Glue	Mice	2015	15
	Suction	Rabbits	1993	67		Glue	Mice	2017	68
	Suction	Rabbits	1994	69	LPS inoculation	Prolonged apnea	Mice	2012	70
	Suction	Dogs	1994	71		Suction	Mice	2014	72
	Suction	Dogs	1995	73		Suction	Mice	2016	74
	Prolonged apnea	Rabbits	1997	75		Suction	Mice	2017	76
	Prolonged apnea	Rabbits	1999	77		Suction	Mice	2019	78
	Suction	Rabbits	2002	79	Cecal ligation and puncture	Suction	Rats	2000	80
	Suction	Rats	2005	81		Suction	Mice	2018	82
	Prolonged apnea	Mice	2013	83		Suction	Mice	2019	78
	Suction	Mice	2017	19	Allergen challenge	Ultra-thin stick objective	Mice	2008	84
Bacterial infection	Glue	Mice	2010	14		Clamping	Mice	2010	10
	Prolonged apnea	Mice	2013	85		Suction	Mice	2012	86
	Motion correction	Mice	2014	87		Suction	Mice	2019	88
	Prolonged apnea	Mice	2014	13	Sickle cell disease	Suction	Mice	2014	89
	Prolonged apnea	Mice	2016	90		Suction	Mice	2017	76
	Suction	Mice	2016	91	Oleic acid inoculation	Window approach	Rats	1994	92
	Suction	Mice	2017	93	Hyperthermia/hypothermia/hypovolemia/ hypoventilation	Prolonged apnea	Rats	2001	94
	Suction	Mice	2017	95	Acid inoculation	Prolonged apnea	Mice	2009	96
	Suction	Mice	2018	97	Phorbol 12-myristate 13-acetate inoculation	Suction	Rats	2011	17
	Suction	Mice	2018	98	Heparinase inoculation	Prolonged apnea	Mice	2012	70
	Suction	Mice	2018	99	Cytokine inoculation	Suction	Mice	2013	100
Viral infection	Suction	Mice	2018	5	Transfusion	Suction	Mice	2015	101
Tumor metastasis	Suction	Mice	2000	102	Thermal hepatic injury	Suction	Mice	2017	103
	Suction	Mice	2015	104	Fibrosis	Suction	Mice	2019	105
	Suction	Mice	2015	106	Cytokine inoculation	Suction	Mice	2019	107
	Suction	Mice	2016	108					
	Suction	Mice	2016	18					
	Suction	Mice	2016	109					
	Glue	Mice	2018	110					

injuries. The information provided will also be useful to those using two-photon imaging analysis for the evaluation of the effects of drugs and vaccines, as well as biological events in the lungs and other organs (e.g., liver, spleen)[5]. Moreover, with minor modifications, the approach could be applied to analyses of other respiratory diseases, including other infectious models (e.g., severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS)), pulmonary fibrosis, and tumor metastasis.

Materials

Biological materials

- *Mice*. We have successfully used 6- to 10-week-old C57BL/6 mice (Japan SLC, mouse line C57BL/6JJmsSlc) and the following transgenic mouse lines: *CAG-ECFP* (cat. no. 004218), *Cd11c-DTR/GFP* (cat. no. 004509), *Zbtb46-GFP* (cat. no. 027618), *Csf1r-GFP* (cat. no. 018549), *Cx3cr1-GFP* (cat. no. 005582), *Ncr1-GFP* (cat. no. 022739), *Clec9a-GFP* (cat. no. 017696), *Sftpc-GFP* (cat. no. 028356), *Cd11c-Cre* (cat. no. 008068), *Zbtb46-Cre* (cat. no. 028538), *Cx3cr1-Cre* (cat. no. 025524), *Cx3cr1-CreER* (cat. no. 020940), *Cd8a-Cre* (cat. no. 008766), *Cd4-CreER* (cat. no. 022356), *Cd19-Cre* (cat. no. 006785), *Mcpt8-Cre* (cat. no. 017578), *loxP*-flanked *R26-tdTomato* (Ai14) (cat. no. 007914), *R26-EYFP* (cat. no. 006148), and *R26-mT/mG* (cat. no. 007676) mice, which can be obtained from the Jackson Laboratory. *CAG-Cre* mice can be obtained from J. Miyazaki (Osaka University Graduate School of Medicine)[29]. *LysM-GFP* mice can be obtained from T. Graf (Albert Einstein College of Medicine)[30]. *Sftpc-CreER* mice can be obtained from B.L.M. Hogan (Duke University Medical Center)[31]. *Ly6g-Cre* (Catchup) mice can be obtained from M.G.[32]. *R26-mTFP1* mice can be obtained from I. Imayoshi (Kyoto University)[33]. Cre strains were bred to *R26-tdTomato*, *R26-EYFP*, *R26-mTFP1*, or *R26-mT/mG* mice. $Sftpc^{CreER/+}$;$R26^{tdTomato/+}$ mice, $Sftpc^{CreER/+}$;$R26^{mTmG/+}$ mice, $Sftpc^{CreER/+}$;$R26^{tdTomato/+}$;$Cx3cr1^{GFP/+}$mice and $Cx3cr1^{CreER/+}$;$R26^{tdTomato/+}$ mice were intraperitoneally injected with 1 mg of tamoxifen for 5 d. $Cd4^{CreER/+}$;$R26^{tdTomato/+}$ mice and $Cd4^{CreER/+}$;$R26^{mTmG/+}$ mice were intraperitoneally injected with 5 mg of tamoxifen for 5 d **! CAUTION** All animal care and experiments must conform to the guidelines for animal experiments of the relevant government and institution. All our animal care and experiments conformed to the guidelines for animal experiments of the University of Tokyo and were approved by the animal research committee of the University of Tokyo (PA17-31 and PA17-17).
- *Viruses*. We have used MA-eCFP-H5N1, MA-Cerulean-H5N1, MA-eGFP-H5N1, MA-Venus-H5N1, and MA-mCherry-H5N1 (A/Vietnam/1203/2004[H5N1]); and MA-eCFP-PR8, MA-Cerulean-PR8, MA-eGFP-PR8, MA-Venus-PR8, and MA-mCherry-PR8 (A/Puerto Rico/8/34[H1N1]), which express a fluorescent reporter protein (eCFP, Cerulean, eGFP, Venus, or mCherry) fused to the NS1 protein. Viruses were generated by using reverse genetics[25–27]. Virus strains should be propagated in Madin-Darby canine kidney (MDCK) cells. The MDCK cell line we used was obtained from R.G. Webster (St. Jude Children's Research Hospital). DNA fingerprinting showed that this cell line has the same origin as one obtained from ATCC (cat. no. CCL-34, RRID:CVCL_0422) **! CAUTION** All viruses and infected animals should be handled in accordance with your institution's biosafety regulations. All work on highly pathogenic avian influenza viruses must be performed under biosafety level 3 (BSL3) regulations. Accordingly, all our in vivo imaging studies were performed in the BSL3 facility at the University of Tokyo (Tokyo, Japan), which is approved for such use by the Ministry of Agriculture, Forestry, and Fisheries of Japan **▲ CRITICAL** The cells should be regularly checked to ensure that they are not contaminated with mycoplasma.

Reagents

▲ CRITICAL Although the suppliers used for all reagents are provided, alternative reagents are available in most cases. All reagents should be stored according to the manufacturer's recommendations. For aliquot sizes for reagents, see the 'Reagent setup' section.

- Sterile phosphate buffered saline (PBS, pH 7.4; made in-house)
- Sterile saline solution (NaCl, 0.9% (wt/vol); made in-house)
- Dimethyl sulfoxide, sterile-filtered (DMSO; Nacalai Tesque, cat. no. 13408-64) **! CAUTION** DMSO readily penetrates the skin; wear rubber gloves and protective eye goggles.
- Sunflower seed oil (Sigma-Aldrich, cat. no. 88921)
- Ethanol (99.5%; FujiFilm Wako Pure Chemical, cat. no. 057-00456) **! CAUTION** Ethanol is highly flammable and may cause eye irritation. Handle it appropriately.
- Tamoxifen (Sigma-Aldrich, cat. no. T5648)

- Isoflurane (MSD Animal Health) **! CAUTION** Isoflurane is an anesthetic gas associated with adverse health outcomes. It should be used in a well-ventilated room or with another appropriate removal system. Store it in a locked drawer at room temperature (18–25 °C).
- Sevoflurane (Maruishi Pharmaceutical) **! CAUTION** Sevoflurane is an anesthetic gas associated with adverse health outcomes. It should be used in a well-ventilated room or with another appropriate removal system. Store it in a locked drawer at room temperature.

Fluorescent reagents **! CAUTION** Fluorescent reagents can be harmful. They should be handled according to the manufacturer's instructions while wearing proper protective clothing **▲ CRITICAL** Choose fluorescent reagents as required for your experiment.

- Cascade Blue-conjugated dextran (10,000 molecular weight (MW); Invitrogen, cat. no. D1976)
- Fluorescein isothiocyanate (FITC)-conjugated dextran (4,000 MW; Sigma-Aldrich, cat. no. 46944)
- FITC-conjugated dextran (10,000 MW; Invitrogen, cat. no. D1820)
- FITC-conjugated dextran (40,000 MW; Invitrogen, cat. no. D1845)
- FITC-conjugated dextran (70,000 MW; Sigma-Aldrich, cat. no. 46945)
- Texas Red-conjugated dextran (3,000 MW; Invitrogen, cat. no. D3328)
- Texas Red-conjugated dextran (10,000 MW; Invitrogen, cat. no. D1863)
- Texas Red-conjugated dextran (70,000 MW; Invitrogen, cat. no. D1864)
- Qtracker 655 vascular labels (Invitrogen, cat. no. Q21021MP)
- Qdot 655 wheat germ agglutinin (WGA) conjugate (Invitrogen, cat. no. Q12021MP)
- Calcein AM solution (Sigma-Aldrich, cat. no. C1359)
- SYTOX Blue nucleic acid stain (Invitrogen, cat. no. S11348)
- SYTOX Green nucleic acid stain (Invitrogen, cat. no. S7020)
- SYTOX Orange nucleic acid stain (Invitrogen, cat. no. S11368)
- Propidium iodide (Invitrogen, cat. no. P1304MP)
- DAPI (4′,6-diamidino-2-phenylindole, dilactate; Invitrogen, cat. no. D3571)
- Hoechst 33342, trihydrochloride, trihydrate (Invitrogen, cat. no. H3570)
- Pan caspase (FAM-VAD-FMK) in vivo probe, green (Vergent Bioscience, cat. no. 20100)
- CellROX Green Reagent (Invitrogen, cat. no. C10444)
- CellROX Orange Reagent (Invitrogen, cat. no. C10443)
- CellROX Deep Red Reagent (Invitrogen, cat. no. C10422)
- LysoTracker Blue DND-22 (Invitrogen, cat. no. L7525)
- LysoTracker Green DND-26 (Invitrogen, cat. no. L7526)
- LysoTracker Red DND-99 (Invitrogen, cat. no. L7528)
- LysoTracker Deep Red (Invitrogen, cat. no. L12492)
- MitoTracker Orange CMTMRos (Invitrogen, cat. no. M7510)
- MitoTracker Red CM-H_2Xros (Invitrogen, cat. no. M7513)
- MitoTracker Red FM (Invitrogen, cat. no. M22425)
- Rhodamine 6G (Sigma-Aldrich, cat. no. 252433)
- Tetramethylrhodamine, ethyl ester, perchlorate (TMRE; Invitrogen, cat. no. T669)
- FluoSpheres polystyrene microspheres (1.0 μm, red fluorescent; Invitrogen, cat. no. F13083)
- SiR-actin (Cytoskeleton, cat. no. CY-SC001)
- SiR-tubulin (Cytoskeleton, cat. no. CY-SC002)
- PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich, cat. no. PKH26PCL)
- FITC-conjugated anti-mouse Ly-6G antibody (BioLegend, cat. no. 127606, RRID: AB_1236494)
- Alexa Fluor 488–conjugated anti-mouse Ly-6G antibody (BioLegend, cat. no. 127626, RRID: AB_2561340)
- DyLight 488–conjugated anti-mouse Ly-6G antibody (Leinco Technologies, cat. no. L287, RRID: AB_2810281)
- PE-conjugated anti-mouse Ly-6G antibody (BD Biosciences, cat. no. 551461, RRID: AB_394208)
- Alexa Fluor 594–conjugated anti-mouse Ly-6G antibody (BioLegend, cat. no. 127636, RRID: AB_2563207)
- Alexa Fluor 647–conjugated anti-mouse Ly-6G antibody (BioLegend, cat. no. 127610, RRID: AB_1134159)

Equipment

- Dark microtubes, (1.5 ml; Watson, cat. no. 131-915)
- Microsurgery straight scissors (13.5 cm; BRC, cat. no. 64152075)
- Microsurgery straight iris scissors (11.0 cm; BRC, cat. no. 64122001)
- Microsurgery hooked forceps (12.7 cm; BRC, cat. no. 64121044)
- Microsurgery bulldog forceps (BRC, cat. no. 70052-30CII/R)
- Tracheal cannula (1.1 × 32 mm; i.d., 0.80 mm; Nipro, cat. no. 09-043)
- Insulin syringes (0.5 ml, 100 U, 30 gauge × 10 mm; Nipro, cat. no. 08277)
- Pasteur pipettes (BD Falcon, cat. no. 357575)
- Customized surgical retractor (made in-house)
- Thoracic suction window (Sakura Seiki, custom made)
- Stage for mounting a thoracic suction window (Sakura Seiki, custom made)
- Suction regulator (Iwaki, cat. no. 1450050)
- Cover glass (Matsunami Glass, cat. no. C013001)
- Hot plate (Hipet, cat. no. 4977007036379)
- Adhesive tape (Yamato, cat. no. NO200-19)
- Customized microscope stage (Narishige, custom made)
- Confocal microscope system (Zeiss, model no. LSM 780 NLO)
- Infrared laser (Coherent, model no. Chameleon Vision II)
- 20× water immersion lens (Zeiss, Plan-Apochromat model)
- Beam-pointing stabilizer (TEM Messtechnik, model no. Aligna 4D system)
- High-efficiency particulate air (HEPA) filters (Vacushield; Pall, cat. no. 4402)
- Artificial ventilator (Shinano, cat. no. SN-480-7)
- Airway pressure monitor (Shinano)
- Gas anesthesia vaporizer (Shinano, cat. no. SN-487-OT)
- Mouse anesthesia induction chamber (Shinano, cat. no. SN-487-85-02)
- Mouse anesthesia mask (Shinano, cat. no. SN-487-70-08)
- Parafilm (Laboratory & Medical Supplies, cat. no. PM-996)
- Positive pressure mask (Versaflo Faceshields; 3M, cat. no. TR-300-HKL and TR-3712N)
- Tyvek suit (DuPont, cat. no. SoftWear III)
- Surgical gloves (SIAM OKAMOTO, cat. no. OM-100)
- Small glass window (Thorlabs, cat. no. WG12012-B)
- Planar window, RS seal (Roxtec, cat. no. RS 100 AISI 316 woc/SLFRS 100 AISI 316)
- Pulse oximeter (Kent Scientific, model. no. LabOx-1)

Software

- CellProfiler (Broad Institute: https://cellprofiler.org/)
- MATLAB (MathWorks: https://www.mathworks.com/products/matlab.html)
- Prism 6 software (GraphPad: https://www.graphpad.com/scientific-software/prism/)
- ImageJ (NIH: https://imagej.nih.gov/ij/)
- TrackMate[34], a plugin for ImageJ (NIH: https://imagej.net/TrackMate)

Reagent setup

▲ **CRITICAL** All reagents should be prepared under sterile conditions. Fluorescent reagents should be protecting from light during the setup procedure because they are light sensitive.

Tamoxifen solution

To prepare 10 mg/ml of tamoxifen solution in sunflower seed oil, dissolve 100 mg of tamoxifen in 1 ml of ethanol (99.5%) and add 9 ml of sunflower seed oil. After adding the ethanol and sunflower seed oil, mix well by vortexing and sonication. This solution can be stored in a refrigerator (2–8 °C) for a week. **! CAUTION** Tamoxifen powder should be handled in a hood. To avoid inhalation and contact with skin, wear rubber gloves and a surgical mask.

Fluorescent dextran

Prepare a solution at a concentration of 2 mg/ml in sterile 1× PBS or saline, make aliquots in 1.5-ml microtubes, and store them in a refrigerator (2–8 °C) for up to 2 weeks. Inject 50 μl (100 μg) of fluorescent dextran i.v. per mouse.

Qtracker 655 vascular labels
Immediately before use, add 5 μl of the stock solution to 95 μl of sterile 1× PBS or saline to make 100 μl total and inject 50 μl i.v. at a concentration of 0.1 μM.

FluoSpheres polystyrene microspheres
Prepare a solution at a concentration of 1×10^8 beads/ml in sterile 1× PBS or saline, make aliquots of the solution in dark 1.5-ml microtubes, and store them in a refrigerator (2–8 °C) for long periods (~3 months). Immediately before use, mix well and inject 50 μl i.v. per mouse.

Qdot 655 WGA
Immediately before use, add 5 μl of the stock solution to 95 μl of sterile 1× PBS or saline to make 100 μl total and i.v. inject 50 μl.

Calcein AM solution
Prepare a solution at a concentration of 100 μM in sterile 1× PBS or saline, dispense the solution into dark 1.5-ml microtubes, and store them in a refrigerator (2–8 °C) for up to 2 weeks. Inject 50 μl of fluorescent dextran i.v. per mouse.

SYTOX Blue, Green, and Orange
Divide the 5 mM DMSO stock solution into dark 1.5-ml microtubes and store them at −20 °C for up to 3 months. Immediately before use, prepare a solution at a concentration of 50 μM in sterile 1× PBS or saline and i.v. inject 50 μl per mouse.

Propidium iodide
Prepare a solution at a concentration of 100 mM in sterile 1× PBS or saline, dispense the solution in dark 1.5-ml microtubes, and store them at −20 °C for up to 3 months. Immediately before use, prepare a solution at a concentration of 1 mM in sterile 1× PBS or saline and inject 50 μl i.v. per mouse.

DAPI
Prepare a solution at a concentration of 10 mM in sterile 1× PBS or saline, make aliquots of the solution in dark 1.5-ml microtubes, and store them in a refrigerator (2–8 °C) for up to 2 weeks. Inject 50 μl of the solution i.v. per mouse.

Pan caspase (FAM-VAD-FMK) in vivo probe
Prepare a working solution according to the vendor's manual, dissolve pan caspase in vivo probe in 5 μl of DMSO, and add 55 μl of 1× injection buffer (from the kit). Inject 60 μl of the solution i.v. per mouse within 1 h of preparation.

PKH26
Prepare a working solution according to the vendor's manual, dissolve 100 μl of PKH26PCL in 900 μl of ethanol and store at room temperature for up to 3 months. Immediately before use, prepare a solution at a concentration of 10 μM in sterile Dilution Buffer (from the kit) and inject 50 μl intranasally per mouse.

CellROX Green, Orange, and Deep Red
Immediately before use, add 50 μl of the stock solution to 450 μl of sterile 1× PBS or saline to make 500 μl total and inject 50 μl i.v. at a concentration of 250 μM.

LysoTracker Blue, Green, Red, and Deep Red
Immediately before use, add 50 μl of the stock solution to 450 μl of sterile 1× PBS or saline to make 500 μl total and inject 50 μl i.v. at a concentration of 100 μM.

MitoTracker Orange CMTMRos, Red CM-H2Xros, and Red FM
Immediately before use, dilute 50 μg of MitoTracker in 1 ml of DMSO and inject 50 μl i.v. at a concentration of 100 μM. ▲CRITICAL The MitoTracker solution should be prepared fresh each time immediately before use.

Rhodamine 6G

Prepare the solution at a concentration of 10 mM in sterile 1× PBS or saline, make aliquots in dark 1.5-ml microtubes, and store them in a refrigerator (2–8 °C) for up to 2 weeks. Immediately before use, prepare a solution at a concentration of 10 μM in sterile 1× PBS or saline and inject 50 μl i.v. per mouse.

TMRE

Prepare the solution at a concentration of 10 mM in DMSO, make aliquots in dark 1.5-ml microtubes, and store them in a refrigerator (2–8 °C) for up to 2 weeks. Immediately before use, prepare a working solution at a concentration of 1 mM in sterile 1× PBS or saline and inject 50 μl i.v. per mouse.

SiR-actin and SiR-tubulin

Prepare each solution at a concentration of 1 mM in DMSO, make aliquots in dark 1.5-ml microtubes, and store them in a refrigerator (2–8 °C) for up to 1 week. Immediately before use, prepare solutions at a concentration of 100 μM in sterile 1× PBS or saline and inject 50 μl i.v. per mouse.

Fluorescent antibody

Dilute fluorescent antibodies to a concentration of 1 μg per 10 μl with sterile 1× PBS or saline and inject 50 μl i.v. per mouse. **! CAUTION** It should be noted that antibody staining may affect the target cell behavior; for example, at a high dose (~200 μg), antibodies may neutralize cell activities and/or cause antibody-dependent cytotoxic activity[35–37]. In our studies, we use 5 μg of antibody for brightness screening because inoculation of fluorochrome-conjugated anti-Ly-6G antibody at low doses (1–40 μg) into mice does not affect neutrophil recruitment[38]. The contribution of Ly-6G, which is expressed predominantly on murine neutrophils, to recruitment during inflammation remains a matter of debate. It has been reported that low-dose antibody treatment inhibited Ly-6G ligation and the recruitment of neutrophils to the site of inflammation[39]; however, a more recent study indicated that Ly-6G knockout did not affect either neutrophil differentiation or recruitment to the site of inflammation in Catchup mice[32]. Therefore, a low dose of anti-Ly-6G antibody is used in our protocol.

Equipment setup

Laser path adjustment system

An overview of the laser path adjustment system is shown in Fig. 1. Our two-photon excitation laser (Chameleon Vision II) unit is placed on an anti-vibration table outside the BSL3 facility. The laser beam enters the BSL3 room, where the two-photon excitation scanning microscope is located, through a window (composed of a small glass window (WG12012-B) and a planar window (RS seal)) connecting the inside and the outside of the BSL3 facility (Fig. 1c,d). The laser path connecting the laser source unit and the two-photon excitation microscope is adjusted by automated laser beam alignment and the Aligna 4D stabilization system is adjusted with two active mirrors. **! CAUTION** This system adjusts the laser path passing from the outside to the inside of the BSL3 facility for maintenance purposes, so there is no need for this setup unless you are using pathogens that require BSL3 containment. Heat is generated when the laser source unit is running, so keep the temperature and humidity constant by using air conditioning equipment. **! CAUTION** The system should be operated only by users trained to deal with unenclosed high-power invisible beams and should be placed in an appropriate enclosure with interlocking doors.

Two-photon excitation laser scanning microscopy system for in vivo imaging of virus-infected mouse lungs in a BSL3 facility

A schematic of the arrangement of the in vivo lung imaging system for virus-infected mouse is shown in Fig. 2a, and layout examples are shown in Fig. 2b–g. This in vivo lung imaging system is based on the upright microscope LSM 780 NLO system, which is equipped with four different lasers (excitation at 405, 488, 543, and 633 nm) for confocal imaging and a two-photon excitation laser (excitation at 630–1,050 nm). To be able to perform the surgical procedure on the mouse, we replaced the sample stage with a large, flat one (microscope stage for in vivo experiment) as shown in Fig. 2b,c. To efficiently excite multiple fluorescent proteins and fluorescent dyes simultaneously, the wavelength of the infrared laser should be set at 910 nm. All fluorescent spectra between the 410- and 695-nm wavelengths can be detected using a 20× water immersion lens, and we record signals in lambda image stacks (0.13 frames per s, 1,024 × 1,024 pixels) and acquire *z*-stack images with *z*-depths of

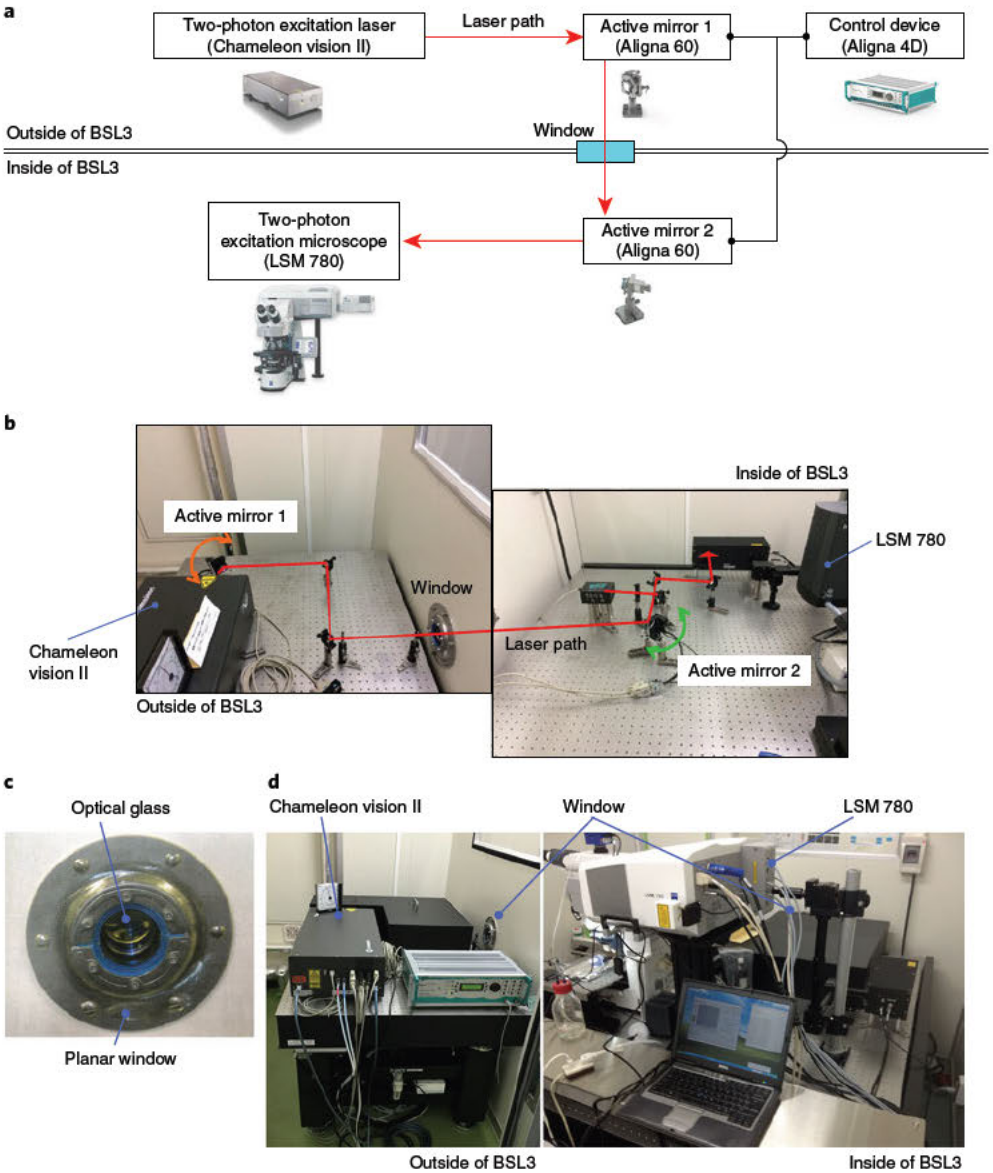

Fig. 1 | The laser path adjustment system. a, Schematic image of the system for correcting the laser beam path. **b**, Layout of active mirrors to adjust the laser path. **c**, The window through which the laser connecting the inside and outside of the BSL3 facility passes. **d**, Arrangement of the two-photon excitation microscope inside the BSL3 facility and the laser unit outside the BSL3 facility. Some images provided courtesy of Coherent and Zeiss.

5 μm (total of 10-μm *z*-depth). We perform spectral separation of the acquired lambda stacks by using the linear unmixing function of the ZEN software. Although the LSM 780 microscope system is controlled by a primary personal computer, we recommended adding >64 GB of RAM for appropriate imaging analysis.

We keep the mice on a heated stage on the sample stage and record their vital signs using a LabOx-1 pulse oximeter. To observe the lungs of the mice with a thoracotomy, we place the ventilator with an airway pressure monitor and anesthesia machine for rodents in appropriate positions on the stage. We installed high-efficiency particulate air (HEPA) filters in the exhalation duct of the ventilation system (Fig. 2b,d), and the operator wore a positive pressure mask (Versaflo Faceshields) and a Tyvek suit (Fig. 2e–g) to avoid exposure to the viruses. **! CAUTION** The wavelength and power of the excitation laser should be adjusted appropriately according to the experimental conditions. Increasing the laser power enhances target signals and enables detection of second-harmonic generation (SHG), in which structures with repeating patterns lead to the formation of a signal. SHG is a useful phenomenon for visualizing collagen fibers in the lung without staining; however, it should be noted that the

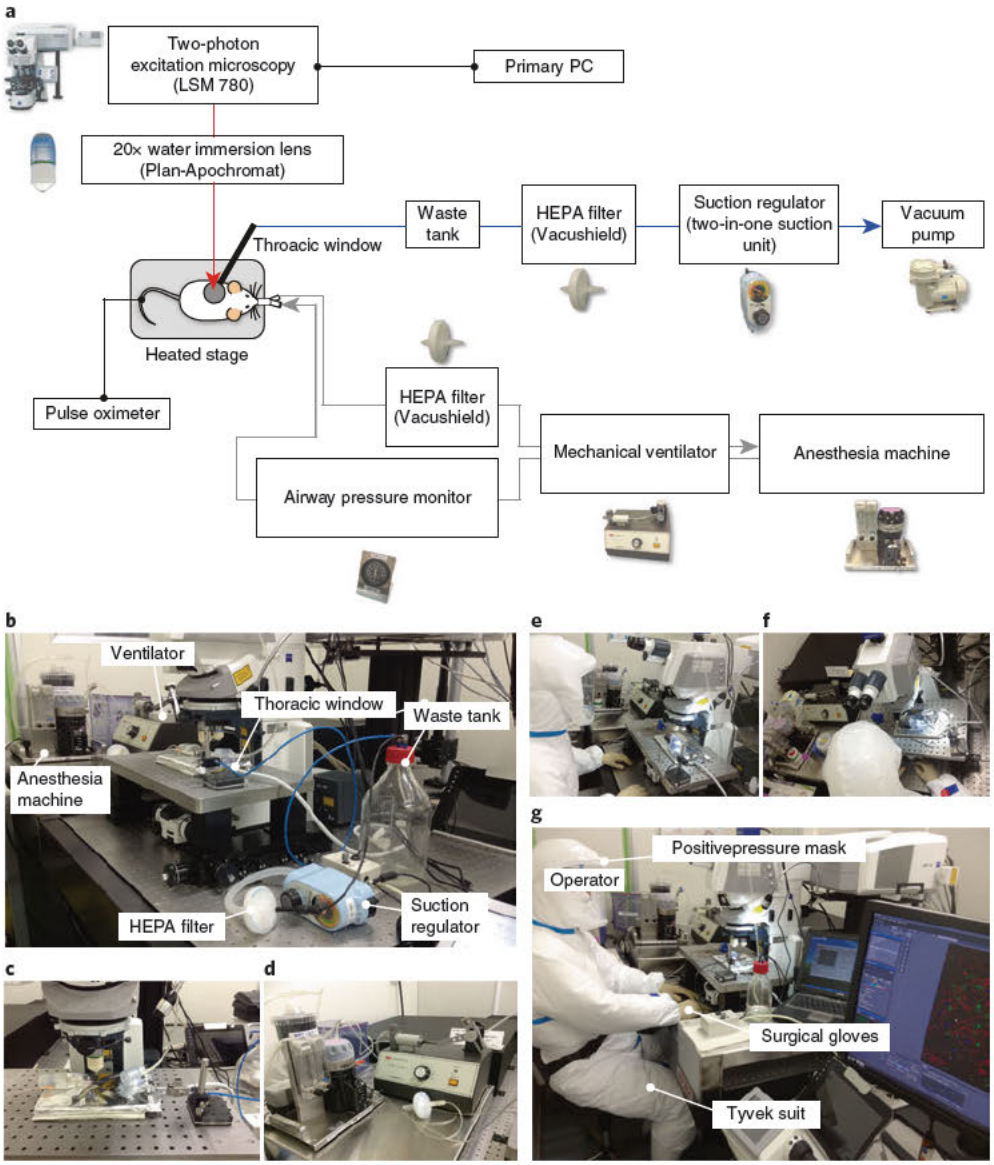

Fig. 2 | The in vivo lung imaging system for virus-infected mouse. a, Schematic image of the imaging system for virus-infected lungs. **b**, Placement of life support devices and lung stabilizer devices. **c**, Surgical stage. **d**, Anesthesia machine and mechanical ventilator. **e–g**, The operator wearing a Tyvek suit and a positive-pressure mask. All our animal care and experiments conformed to the guidelines for animal experiments of the University of Tokyo and were approved by the animal research committee of the University of Tokyo (PA17-31 and PA17-17). Some images in **a** provided courtesy of Zeiss.

autofluorescence of lung tissue is also enhanced under excessive excitation conditions (Supplementary Fig. 1). When using this protocol, we did not perform experiments under which SHG occurs, in order to minimize autofluorescence; it is better to adjust the laser power according to the experimental purpose. When the wavelength of the excitation laser is too short, the autofluorescence signal becomes very strong and it is difficult to observe properly. By contrast, when the laser wavelength is too long, it becomes difficult to obtain a signal because of the short excitation energy (Supplementary Fig. 2). **! CAUTION** Although color separation of emission using a conventional optical band-pass filter is also available for this protocol, multispectral imaging is a useful approach for simultaneously analyzing multiple targets by eliminating tissue autofluorescence and identifying fluorescent labels with overlapping spectra[40,41]. In vivo two-photon imaging is performed under conditions of single stimulation with a two-photon excitation laser; limitations exist regarding available fluorescent reagents/proteins for multiple labeling of target cells and lung architecture. Therefore, we recommend using a multispectral approach to produce crosstalk-free images of fluorescence with overlapping spectra that cannot be

separated by using band-pass filters. Before starting experiments, it is necessary to collect spectral signatures of the emission signal of each fluorescent reagent and protein as reference spectra under the same excitation condition as will be used in the experiment.

Thoracic suction window and surgical tools

To observe the mouse lung using an upright microscope, it is necessary to prepare a thoracic suction window to immobilize the lung. In the BSL3 facility, animal experiments must be performed while wearing two or three layers of latex gloves; therefore, the thoracic suction window was designed for easy handling, even in the BSL3 facility, and to be minimally invasive for the infected animals (Fig. 3a–c and Supplementary Fig. 3). To position a cover glass for each observation, flatten the upper surface of the thoracic suction window so that a commercially available cover glass will fit. This device is also designed to reduce concavity and convexity as much as possible so that blood containing virus cannot accumulate. Connect the thoracic suction window to an aspirator through a waste tank and a suction regulator. To prevent the spread of virus-containing aerosols, install HEPA filters between the waste tank and the suction regulator as shown in Fig. 3d.

Procedure

Infection with fluorescent influenza viruses ● Timing 10–20 min

1 On Day 0, intranasally inoculate C57BL/6 ('B6') mice or transgenic mice with 10^5 plaque-forming units (PFUs) of Color-flu viruses in 50 μl of PBS under sevoflurane anesthesia. Tables 2 and 3 show the brightness levels of fluorescence of representative reporter mouse immune cells and Color-flu viruses in vivo.

! CAUTION All relevant guidelines regarding the use of animals and recombinant viruses should be followed.

▲ CRITICAL Fluorescent reporter influenza viruses (Color-flu) stably express high levels of a reporter protein in the infected cells and show comparable virulence to those of wild-type influenza viruses in mice[25]. Depending on the experiments, modify the virus infectious dose, monitor the infected mice in the days following infection, and determine the appropriate time point for observation (e.g., when mice are infected with 10^3 PFU of MA-Venus-PR8, infected cells can be observed for up to 7 d post-infection). Of note, infected cells may not be observed if the infectious dose is too low.

▲ CRITICAL As controls for the experiment, use wild-type mice or transgenic mice that are not infected with influenza virus and administer the same fluorescently labeled antibodies and reagents as those used in the test group.

Starting up the imaging system equipment ● Timing 20–30 min

2 On the day of analysis, turn on the two-photon excitation laser and the Aligna 4D control unit placed outside the BSL3 facility, and verify that they are working.

▲ CRITICAL The Aligna 4D control unit needs to be kept ON.

3 Wearing a Tyvek suit, positive pressure mask, and gloves according to the guidelines for the BSL3 facility, enter the BSL3 facility where the imaging system is housed.

? TROUBLESHOOTING

4 Turn on the microscope controllers, confocal lasers, and the computer for the two-photon excitation microscope and the Aligna 4D system.

5 Launch the microscope control software ZEN and turn on the lasers, including the two-photon excitation laser.

6 Launch the Aligna 4D control software Kangoo and adjust the laser path connecting the laser source unit and the microscope (Supplementary Fig. 4).

? TROUBLESHOOTING

7 Wrap the hot plate with aluminum foil, turn it on, and keep it at 35 °C. Sterilize the surgical area and tools with 70% ethanol and place all instruments within easy reach.

Animal anesthesia ● Timing 2–3 min

8 Turn on the gas anesthesia vaporizer and supply 5% isoflurane to a mouse anesthesia induction chamber.

9 Anesthetize the influenza virus–infected mouse with 5% isoflurane in a mouse anesthesia induction chamber. Subsequently, transfer the mouse to the hot plate while supplying 2% isoflurane via an anesthetic mask.

? TROUBLESHOOTING

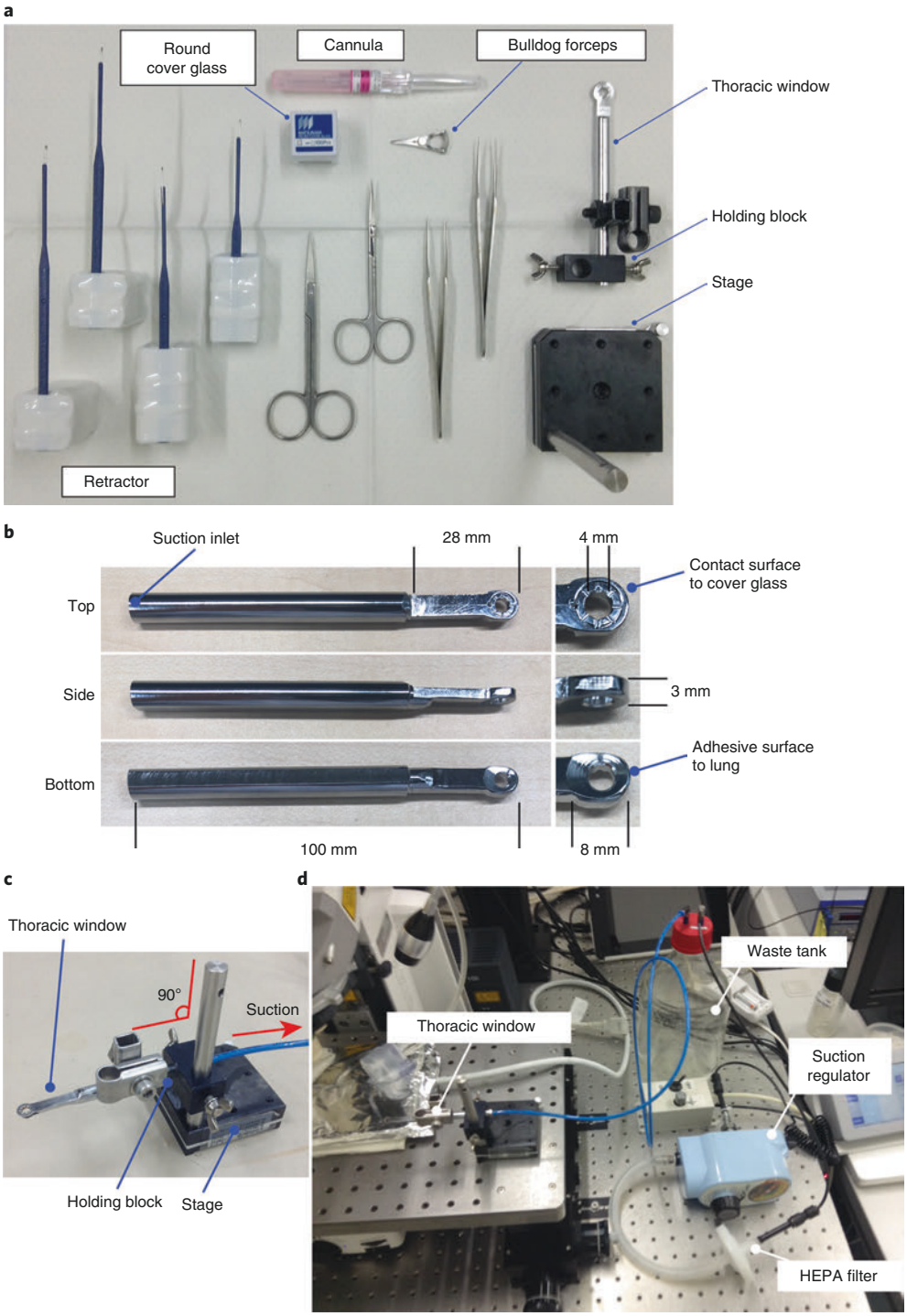

Fig. 3 | Devices to stabilize lungs. a, Surgical tools**. b**, Thoracic suction window. **c**, Setup of thoracic suction window and the holding devices. **d**, Device layout pertaining to lung stabilization.

Administration of fluorescent dyes ● Timing 5 min

10 Inject the chosen fluorescent dyes and antibodies via the retro-orbital plexus (as shown in Supplementary Video 1) using an insulin syringe. Tables 4 and 5 show the brightness levels of antibodies and fluorescence of dyes, respectively, in vivo.

! CAUTION When working with viruses in a BSL3 containment, it is not safe to use needles, so we avoid them as much as possible, which is a standard precaution in high-containment laboratories.

Table 2 | Comparison of fluorescent reporter mice for in vivo imaging using two-photon excitation microscopy

Mouse	Published specificity	Ref.	Brightness	Note
$CAG^{ECFP/ECFP}$	Ubiquitous	111	+++	Fluorescent signals are detectable; useful
$CAG^{Cre/+};R26^{EYFP/+}$	Ubiquitous	29,112	+	Fluorescent signals are hardly detectable
$CAG^{Cre/+};R26^{tdTomato/+}$	Ubiquitous	29,42	++++	Fluorescent signals are very strong
$CAG^{ECFP/+};R26^{mTmG/+}$	Ubiquitous	111,113	++ (ECFP) ++ (mTomato)	Fluorescent signals are detectable; useful
$R26^{mTmG/mTmG}$	Ubiquitous	113	+++	Fluorescent signals are detectable; useful
$Cd11c^{DTR\text{-}GFP/+}$	Dendritic cells	114	+	Fluorescent signals are hardly detectable
$Cd11c^{Cre/+};R26^{EYFP/+}$	Dendritic cells, alveolar macrophages	112,115	+	Fluorescent signals are hardly detectable
$Cd11c^{Cre/+};R26^{tdTomato/+}$	Dendritic cells, alveolar macrophages	42,115	+++	Fluorescent signals are detectable; useful
$Cd11c^{Cre/+};R26^{mTmG/+}$	Dendritic cells, alveolar macrophages (mGFP); ubiquitous other than dendritic cells and alveolar macrophages (mTomato)	113,115	+++ (mGFP) ++ (mTomato)	Fluorescent signals are detectable; useful
$Zbtb46^{GFP/GFP}$	Dendritic cells, endothelial cells	116	+	Fluorescent signals are hardly detectable
$Zbtb46^{Cre/+};R26^{tdTomato/+}$	Dendritic cells, endothelial cells	42,117	++++	Because the fluorescence signal of the endothelial cells is very strong, a bone marrow chimera is needed for immune cell observations
$Zbtb46^{Cre/+};R26^{mTmG/+}$	Dendritic cells, endothelial cells	113,117	++++ (mGFP) ++ (mTomato)	Because the fluorescence signal of the endothelial cells is very strong, a bone marrow chimera is needed for immune cell observations
$Clec9a^{GFP/+}$	Dendritic cells	118	+	Fluorescent signals are hardly detectable
$Csf1r^{GFP/+}$	Macrophages	119	+++	Because many cells are fluorescently labeled, it is difficult to make cell-specific observations, especially in infected lung
$Cx3cr1^{GFP/+}$	Macrophages, monocytes	120	+++	Fluorescent signals are detectable; useful
$Cx3cr1^{Cre/+};R26^{tdTomato/+}$	Macrophages, monocytes	42,121	++++	Because many cells are fluorescently labeled, it is difficult to make cell-specific observations, especially in infected lung
$Cx3cr1^{CreER/+};R26^{tdTomato/+}$	Macrophages, monocytes	42,121	+++	Fluorescent signals are detectable; useful
$LysM^{GFP/+}$	Neutrophils, macrophages	30	+++	Because many cells are fluorescently labeled, it is difficult to make cell-specific observations, especially in infected lung
$Ly6g^{Cre/+};R26^{tdTomato/+}$ (Catchup$^{IVM\text{-}red}$)	Neutrophils	32,42	+++	Fluorescent signals are detectable; useful
$Ly6g^{Cre/+};R26^{mTmG/+}$	Neutrophils (mGFP); ubiquitous other than neutrophils (mTomato)	32,113	+++ (mGFP) ++ (mTomato)	Fluorescent signals are detectable; useful
$Ly6g^{Cre/+};R26^{mTFP1/+}$	Neutrophils	32,33	+++	Fluorescent signals are detectable; useful
$Ly6g^{Cre/+};R26^{tdTomato/+};Cx3cr1^{GFP/+}$	Macrophages, monocytes (GFP); neutrophils (Tomato)		32,42,120	+++ (GFP) +++ (Tomato)
Fluorescent signals are detectable; useful				
$Cd4^{CreER/+};R26^{tdTomato/+}$	CD4T lymphocytes	42,122	+++	Fluorescent signals are detectable; useful
$Cd4^{CreER/+};R26^{mTmG/+}$	CD4T lymphocytes (mGFP); ubiquitous other than CD4T lymphocytes (mTomato)	113,122	+++ (mGFP) ++ (mTomato)	Fluorescent signals are detectable; useful
$Cd8a^{Cre/+};R26^{tdTomato/+}$	CD8T lymphocytes	42,123	+++	Fluorescent signals are detectable; useful
$Cd8a^{Cre/+};R26^{mTFP1/+}$	CD8T lymphocytes	33,123	+++	Fluorescent signals are detectable; useful
$Cd19^{Cre/+};R26^{tdTomato/+}$	B lymphocytes	42,124	+++	Fluorescent signals are detectable; useful
$Mcpt8^{Cre/+};R26^{tdTomato/+}$	Basophils	42,125	+++	Fluorescent signals are detectable; useful
$Ncr1^{GFP/+}$	NK cells	126	++	Fluorescent signals are detectable; useful
$Sftpc^{CreER/+};R26^{tdTomato/+}$	Type II pneumocytes	31,42	++++	Fluorescent signals are detectable; useful
$Sftpc^{CreER/+};R26^{mTmG/+}$	Type II pneumocytes (mGFP); ubiquitous other than Type II pneumocytes (mTomato)	31,113	+++ (mGFP) ++ (mTomato)	Fluorescent signals are detectable; useful
$Sftpc^{CreER/+};R26^{tdTomato/+};Cx3cr1^{GFP/+}$	Macrophages, monocytes (GFP); Type II pneumocytes (Tomato)	31,42,120	+++ (GFP) ++++ (Tomato)	Fluorescent signals are detectable; useful
$Sftpc^{GFP/+}$	Type II pneumocytes	127	++++	Fluorescent signals are detectable; useful

The brightness of each fluorescent protein during in vivo lung imaging was scored as relative fluorescence intensity compared with FluoSpheres fluorescent microspheres as an internal standard. For relative intensities of 0–0.2, 0.2–0.6, 0.6–0.9, and >0.9, the brightness scores are represented as +, ++, +++, and ++++, respectively.

Table 3 | Comparison of fluorescent reporter viruses (Color-flu) for in vivo imaging using two-photon excitation microscopy

Reporter protein	Virus name	Titer	Volume	Excitation (nm)	Emission (nm)	Brightness
eCFP	MA-eCFP-PR8 MA-eCFP-H5N1	10^5 PFU	50 µl	910	477	+
Cerulean	MA-Cerulean-PR8 MA-Cerulean-H5N1	10^5 PFU	50 µl	910	475	+++
eGFP	MA-eGFP-PR8 MA-eGFP-H5N1	10^5 PFU	50 µl	910	507	+++
Venus	MA-Venus-PR8 MA-Venus-H5N1	10^5 PFU	50 µl	910	528	+++
mCherry	MA-mCherry-PR8 MA-mCherry-H5N1	10^5 PFU	50 µl	910	610	+

The brightness of each fluorescent protein during in vivo lung imaging was scored as relative fluorescence intensity compared with FluoSpheres fluorescent microspheres as an internal standard. For relative intensities of 0–0.2, 0.2–0.6, 0.6–0.9, and >0.9, the brightness scores are represented as +, ++, +++, and ++++, respectively.

Table 4 | Comparison of fluorochrome-conjugated antibodies for in vivo imaging using two-photon excitation microscopy

Fluorochrome	Product name	Cat. no.	Clone	Concentration	Volume	Excitation (nm)	Emission (nm)	Brightness
FITC	FITC-conjugated anti-mouse Ly-6G antibody	127606, BioLegend	1A8	100 µg/ml	50 µl	910	519	+
AF 488	AF 488–conjugated anti-mouse Ly-6G antibody	127626, BioLegend	1A8	100 µg/ml	50 µl	910	519	+
Dy Light 488	DyLight 488–conjugated anti-mouse Ly-6G antibody	L287, Leinco Technologies	1A8	100 µg/ml	50 µl	910	518	+
PE	PE-conjugated anti-mouse Ly-6G antibody	551461, BD Biosciences	1A8	100 µg/ml	50 µl	910	578	+++
AF 594	AF 594–conjugated anti-mouse Ly-6G antibody	127636, BioLegend	1A8	100 µg/ml	50 µl	910	617	++
AF 647	AF 647–conjugated anti-mouse Ly-6G antibody	127610, BioLegend	1A8	100 µg/ml	50 µl	910	668	ND

The brightness of each fluorochrome during in vivo lung imaging was scored as relative fluorescence intensity compared with FluoSpheres fluorescent microspheres as an internal standard. For relative intensities of 0–0.2, 0.2–0.6, 0.6–0.9, and >0.9, the brightness scores are represented as +, ++, +++, and ++++, respectively. AF, Alexa Fluor; ND, not detected.

In addition, in the BSL3 facility, animal experiments must be performed wearing two or three layers of latex gloves. Tail-vein administration is a common method; however, it is not easy to perform these procedures with so many layers of gloves. Use tweezers to hold down the mouse to make the administration route. When an infected animal is not used, an administration route can be created via the tail vein or the jugular vein.

? TROUBLESHOOTING

Surgical procedure ● Timing 10–15 min

▲CRITICAL Before experimenting with infected animals, practice the surgical procedures with euthanized animals.

11 Place the mouse on its back and tape the anterior limbs with adhesive tape (Fig. 4a).

12 Using straight scissors, cut the skin beneath the chin in the middle and expose the trachea (Fig. 4b). Insert a tracheal cannula and intubate the mouse to facilitate mechanical ventilation with a ventilator (Fig. 4c). Turn on the ventilator, ventilate the mouse at a respiratory rate of 120 breaths per min, and apply positive-end expiratory pressure (PEEP; ~6 cm H_2O) and a tidal volume of ~0.5 mL. Deliver isoflurane continuously at 2% to maintain anesthesia.

! CAUTION Perform the surgery with care so as not to cut the blood vessels. If bleeding occurs, stop the bleeding with fine bulldog forceps for microsurgery.

13 Place the mouse in the right lateral decubitus position and re-fix its anterior limbs with the tape (Fig. 4d). Make an incision in the skin at the left axilla using straight scissors, straight iris scissors, and hooked forceps (Fig. 4e).

! CAUTION Carefully change the mouse's position in order to avoid cannula drop off.

14 Expose the left lung lobe by surgical intercostal incision between ribs 3 and 4, and keep it exposed by using retractors (Fig. 4f).

Table 5 | Comparison of fluorescent dyes for in vivo imaging using two-photon excitation microscopy

Dye	Target	Cat. no.	Concentration	Volume	Excitation (nm)	Emission (nm)	Brightness
Dextran Cascade Blue, 10,000 MW, lysine fixable	Blood flow	D1976, Invitrogen	25 mg/ml	50 µl	910	420	ND
Fluorescein isothiocyanate–dextran average MW 4,000	Blood flow	46944, Sigma-Aldrich	2 mg/ml	50 µl	910	519	+++
Dextran fluorescein, 10,000 MW, lysine fixable	Blood flow	D1820, Invitrogen	2 mg/ml	50 µl	910	519	+++
Dextran fluorescein, 40,000 MW, lysine fixable	Blood flow	D1845, Invitrogen	2 mg/ml	50 µl	910	519	+++
Fluorescein isothiocyanate–dextran, average MW 70,000	Blood flow	46945, Sigma-Aldrich	2 mg/ml	50 µl	910	519	+++
Dextran Texas Red, 3,000 MW, lysine fixable	Blood flow	D3328, Invitrogen	2 mg/ml	50 µl	910	615	+++
Dextran Texas Red, 10,000 MW, lysine fixable	Blood flow	D1863, Invitrogen	2 mg/ml	50 µl	910	615	+++
Dextran Texas Red, 70,000 MW, lysine fixable	Blood flow	D1864, Invitrogen	2 mg/ml	50 µl	910	615	+++
Qtracker 655 vascular labels	Blood flow	Q21021MP, Invitrogen	0.1 µM	50 µl	910	655	+++
FluoSpheres fluorescent microspheres for tracer studies	Blood flow velocity	F-13083, Molecular Probes	1×10^8 beads/ml	50 µl	910	605	++++
Qdot 655 WGA	Whole cells	Q12021MP, Invitrogen	×20	50 µl	910	655	+
Calcein AM solution	Live cells	C1359, Sigma-Aldrich	100 µM	50 µl	910	520	ND
SYTOX Blue nucleic acid stain	Dead cells	S11348, Invitrogen	50 µM	50 µl	910	480	+++
SYTOX Green nucleic acid stain	Dead cells	S7020, Invitrogen	50 µM	50 µl	910	523	+++
SYTOX Orange nucleic acid stain	Dead cells	S11368, Invitrogen	50 µM	50 µl	910	570	+++
Propidium iodide nucleic acid stain	Dead cells	P1304MP, Invitrogen	1 mM	50 µl	910	617	++++
DAPI nucleic acid stain	Dead cells	D3571, Invitrogen	10 mM	50 µl	910	461	ND
Hoechst 33342	Nuclei	H3570, Invitrogen	10 mg/ml	50 µl	910	461	++
Cas-MAP Green in vivo fluorescent imaging probes	Apoptotic cells	20100, Vergent Bioscience	×1	60 µl	910	533	ND
PKH26 Red Fluorescent Cell Linker Kit for Phagocytic Cell Labeling	Phagocytic cells	PKH26PCL, Sigma-Aldrich	10 µM	50 µl (intranasal administration)	910	567	+++
CellROX Green Reagent	Oxidative stress	C10444, Molecular Probes	250 µM	50 µl	910	520	+++
CellROX Orange Reagent	Oxidative stress	C10443, Molecular Probes	250 µM	50 µl	910	565	+++
CellRox Deep Red	Oxidative stress	C10422, Molecular Probes	250 µM	50 µl	910	665	+
LysoTracker Blue DND-22	Lysosomes	L7525, Molecular Probes	100 µM	50 µl	910	422	ND
LysoTracker Green DND-26	Lysosomes	L7526, Molecular Probes	100 µM	50 µl	910	511	+++
LysoTracker Red DND-99	Lysosomes	L7528, Molecular Probes	100 µM	50 µl	910	590	++
LysoTracker Deep Red	Lysosomes	L12492, Molecular Probes	100 µM	50 µl	910	668	++
MitoTracker Orange CMTMRos	Mitochondria	M7510, Invitrogen	100 µM	50 µl	910	576	+++
MitoTracker CM-H_2Xros	Mitochondria	M7513, Invitrogen	100 µM	50 µl	910	599	+++
MitoTracker Red FM	Mitochondria	M22425, Invitrogen	100 µM	50 µl	910	644	++
Rhodamine 6G	Mitochondria	252433, Sigma-Aldrich	10 µM	50 µl	910	555	+++
TMRE	Mitochondria	T669, Invitrogen	1 mM	50 µl	910	575	+++
SiR-actin	Actin	CY-SC001, SPIROCHROME	100 µM	50 µl	940	674	+
SiR-tubulin	Tubulin	CY-SC002, SPIROCHROME	100 µM	50 µl	940	674	ND

The brightness of each fluorochrome during in vivo lung imaging was scored as relative fluorescence intensity compared with FluoSpheres fluorescent microspheres as an internal standard. For relative intensities of 0–0.2, 0.2–0.6, 0.6–0.9, and >0.9, the brightness scores are represented as +, ++, +++, and ++++, respectively. ND, not detected.

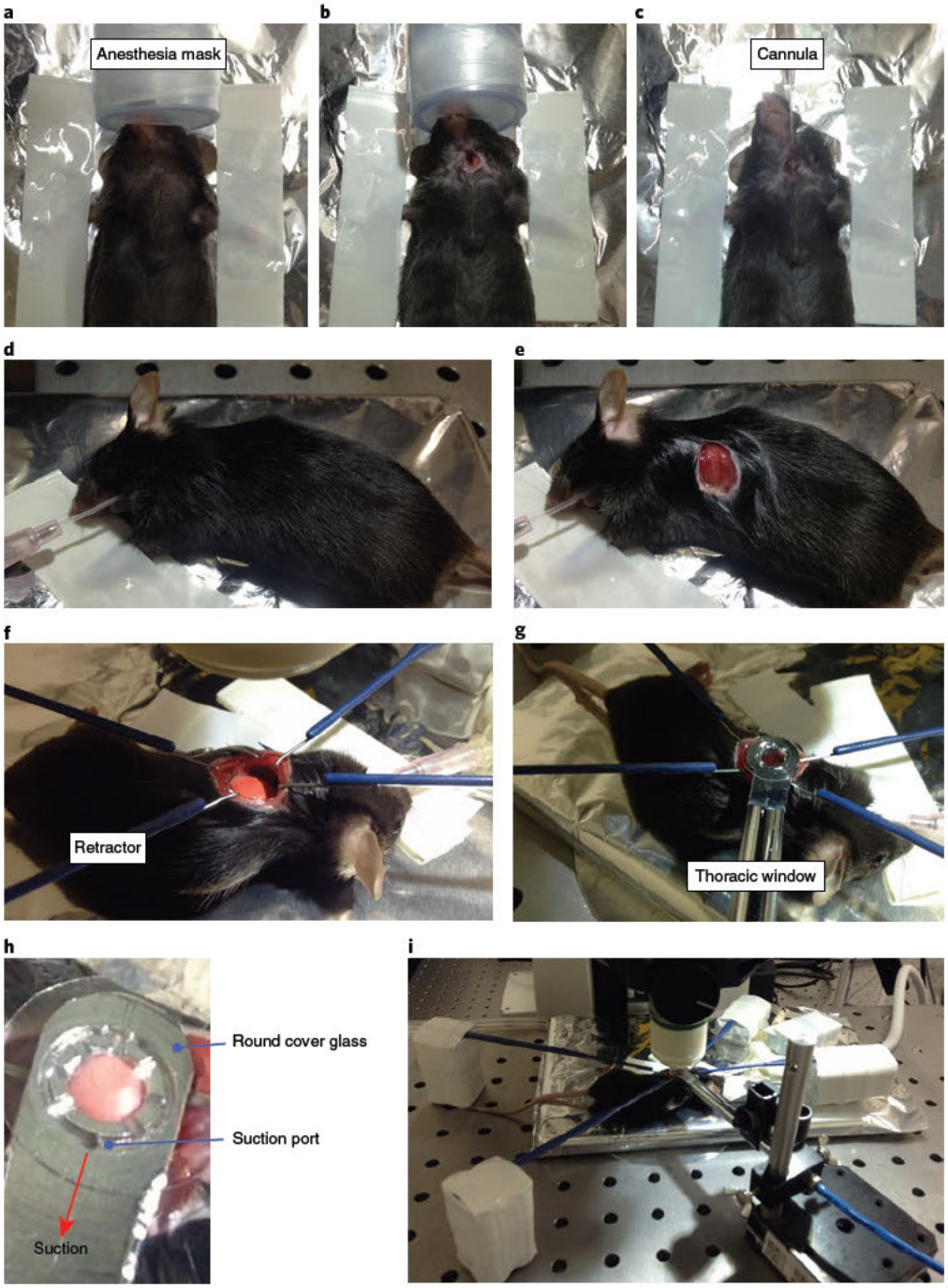

Fig. 4 | Surgical procedure for lung imaging. a, Place the mouse on its back and tape with adhesive tape. **b**, Cut the skin beneath the chin and expose the trachea. **c**, Insert a tracheal cannula. **d**, Place the mouse in the right lateral decubitus position. **e**, Make an incision in the skin at the left axilla. **f**, Expose the left lung lobe and keep it exposed by using retractors. **g**, Lower the thoracic suction window gently to immobilize the lungs of the mouse. **h**, Close-up of the thoracic suction window. **i**, Lower the objective lens to the thoracic suction window. All our animal care and experiments conformed to the guidelines for animal experiments of the University of Tokyo and were approved by the animal research committee of the University of Tokyo (PA17-31 and PA17-17).

! CAUTION Perform the surgery with care so as not to cut the blood vessels. If bleeding occurs, stop the bleeding with fine bulldog forceps for microsurgery.

▲ CRITICAL Because lungs infected with viruses often shrink, secure a large field of surgical view so that the suction window can reach it.

Table 6 | Open-source packages for image processing and analyses

Purpose	Software	Resource	Features	Ref.
Unmixing of lambda image stack	Hyper-Spectral Phasors	https://www.nature.com/articles/nmeth.4134	Windows/macOS executable	128
	Orfeo ToolBox	https://www.orfeo-toolbox.org/	Windows/macOS/Linux executable	129
	Spectral Unmixing Plugins	https://imagej.nih.gov/ij/plugins/spectral-unmixing.html	ImageJ plugin	130,131
	PoissonNMF	https://neherlab.org/poisson_nmf_overview.html	ImageJ plugin	132
Respiratory artifact correction	Imregdemons (image-processing toolbox for MATLAB)	https://www.mathworks.com/help/images/ref/imregdemons.html	MATLAB function	133,134
	Automatic image reconstruction	Algorithm described in the original paper	Algorithm	135
	Intravital microscopy artifact reduction tool (IMART)	http://www.medicine.iupui.edu/icbm/software/	MATLAB executable	136,137
	Intravital Microscopy Toolbox	https://doi.org/10.1371/journal.pone.0053942.s020 or http://stevelacroix.crchudequebec.ca/support-visuel_en.php	ImageJ macro	138
	Galene	https://galene.flimfit.org/	Windows/macOS executable	139
Single-cell tracking	The Tracking Tool (tTt)	https://www.nature.com/articles/nbt.3626	Windows/macOS executable	140
	CellProfiler	https://cellprofiler.org/	Windows/macOS executable	141
	Icy	http://icy.bioimageanalysis.org/	Java application	142
	TrackMate	http://fiji.sc/TrackMate	ImageJ plugin	34

15 Place the mouse beneath the objective lens and connect a device to monitor the heart rate of the mouse (we use a LabOx-1 pulse oximeter).
? TROUBLESHOOTING

Starting up the thoracic vacuum window system ● Timing 2–3 min

16 Turn on the aspirator connected to the thoracic suction window.

17 Fix the thoracic suction window to the holding block at a 90° angle and put a round cover glass on the tip of the suction device.
? TROUBLESHOOTING

18 Turn on the suction pressure regulator and adjust the suction pressure to 25–30 mmHg.

Observation of lungs infected with influenza viruses ● Timing 2–3 min

19 Lower the thoracic suction window gently to immobilize the mouse lungs (Fig. 4g,h). The thoracic suction window should cause the lung to stick to the cover glass because of negative pressure.
! CAUTION Carefully move the suction window so as not to scratch the objective.

20 Position the objective lens above the thoracic window.

21 Put water drops on the cover glass by using a pasteur pipette and lower the objective lens to the thoracic suction window (Fig. 4i).

22 Double-check the general condition of the mouse and its position.

Data acquisition ● Timing 1–4 h per sample

23 Acquire images using the lambda mode of the ZEN software. Record time series at different frequencies according to need.

Unmixing of spectrum data and analyzing the images ● Timing 1–2 h per sample

24 To unmix the spectrum data, prepare a reference image of each spectrum in advance. To make a reference image, acquire each fluorescent dye or protein separately without any co-staining and analyze the single fluorescent spectrum. We use the linear unmixing module of the ZEN software for separating spectrum data; however, other commercial or open-source software is available (Table 6).

25 Subject unmixed time-series stacks to image registration to correct for tissue drifts and respiratory artifacts. This step is critical to certain analyses, such as long-term tracking of individual cells or subcellular structures. In some cases, a reference channel is required for determining the shift and distortion of the objects. In our studies, we use time-series stacks of blood vessels or collagens for such use, because their localizations are constant over time without substantial changes in shape or structure during the observation.

! CAUTION Some image registration algorithms may cause spatial distortion. Choose algorithms that generate corrected data suitable for your subsequent analyses, especially when examination of the shape and structure of cells and tissues is required.

26 Analyze the movies as required for your experiment.

Troubleshooting

Troubleshooting advice can be found in Table 7.

Table 7 | Troubleshooting table

Step	Problem	Possible reason	Solution
3	Difficulty handling mice in BSL3 facility	Normal gloves are not suitable for working in a BSL3 facilities	To perform detailed work in a BSL3 facility, the outermost gloves should be surgical gloves that match the size of your hand
6	No laser signal on the Aligna 4D control software	Laser switch is off	Make sure that the laser switch is turned on with the main unit and the ZEN software
9	Mice die during anesthesia	The level of anesthesia is too high	Decrease the concentration of anesthesia as soon as the mouse shows loss of righting reflex
10	Mice regain consciousness during anesthesia	The level of anesthesia is too low	Confirm the concentration of anesthesia; administer the reagents again after a brief pause
15	No heart rate is measured	The monitoring probe is mispositioned	Make sure that the monitoring probe is in the appropriate place
17	The cover glass falls off	The cover glass does not hold on the suction device	Put water droplets on the tip of the suction device and then place the cover glass on it

Timing

Step 1, infection: 10–20 min
Steps 2–7, starting up the imaging system equipment: 20–30 min
Steps 8–22, anesthesia and surgical preparation for imaging: 21–29 min
Steps 23–26, data acquisition and image analyses: 2–6 h per sample (depending on the number of samples, fluorescent colors, and acquired frames)

Anticipated results

The imaging system described in this protocol enables the observation of the behavior of virus-infected cells and immune cells in infected lungs in real time. Typical images of influenza virus–infected lung are shown in Fig. 5a and Supplementary Video 2. When observing while using a multicolor fluorescent label, it is easier to analyze the detected images if the brightness level of each fluorophore is adjusted to make them similar. It is better to choose fluorescent dyes or proteins that possess high fluorescence stability for long-term observations (Tables 2, 4 and 5). We have found that use of MA-Cerulean-viruses or MA-Venus-viruses for infection produces influenza virus–infected cells with sufficient brightness (Table 3). For labeling immune cells and alveolar cells, we have achieved good results by using the fluorochrome phycoerythrin (PE) for antibody staining and Rosa-tdTomato[42] or -mTFP1[33] mice that were crossed with cell-specific Cre-expressing mice. If using reporter mice expressing a fluorescent protein such as GFP, which is regulated by an endogenous promoter, the expression level of the fluorescent protein should be confirmed. To visualize the lung structure, we use Texas-Red dextran or Qtracker 655 Vascular Labels for the red to far-infrared channel.

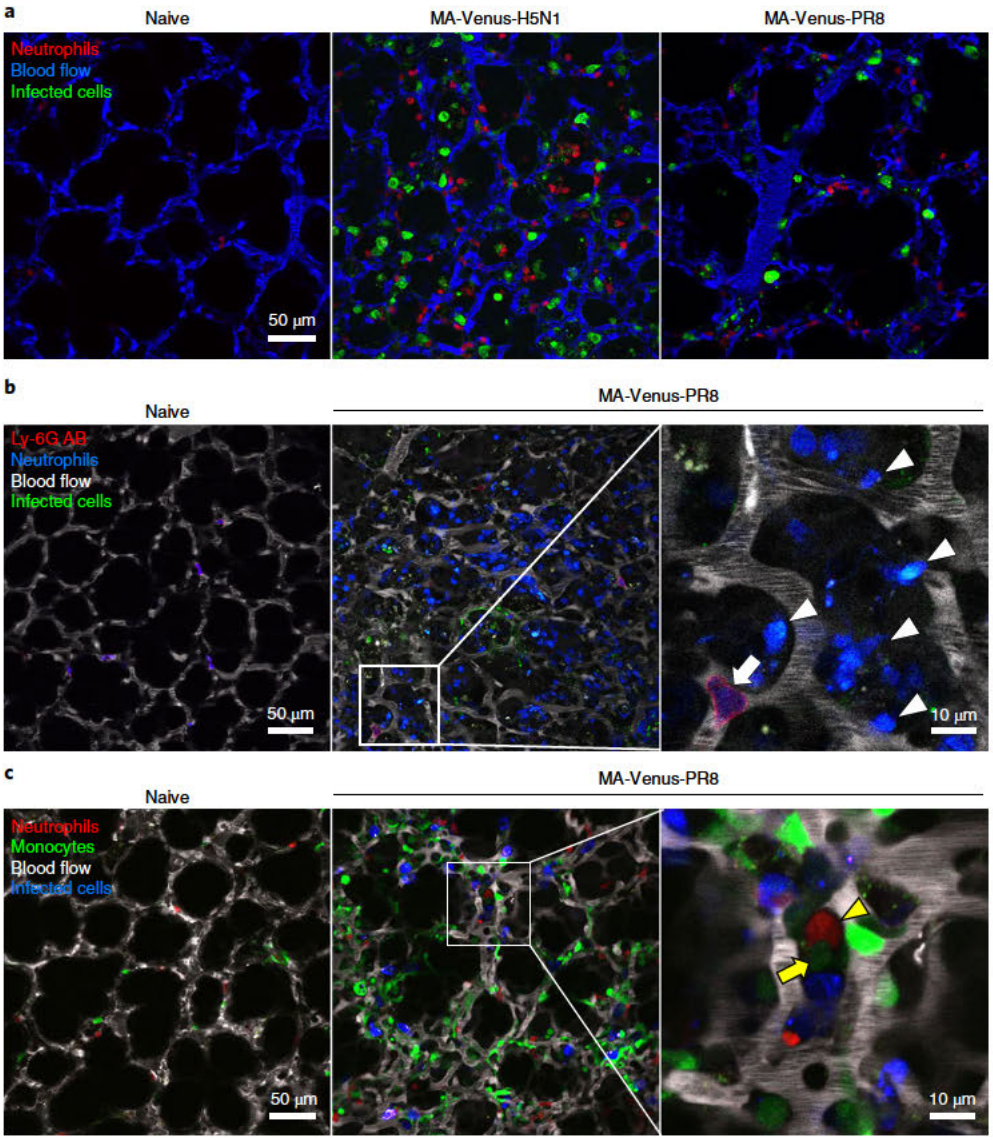

Fig. 5 | In vivo multicolor imaging of influenza virus-infected lungs. a, Catchup$^{IVM\ red}$ mice were intranasally infected with 10^5 PFU of MA-Venus-H5N1 or MA-Venus-PR8 virus and observed at 4 d post-infection. Fluorescent dextran (blue) was intravenously administered to visualize the lung architecture. Red and green indicate neutrophils and virus-infected cells, respectively. **b**, *Ly6g*$^{Cre/+}$;*R26*$^{mTFP1/+}$ mice were intranasally infected with 10^5 PFU of MA-Venus-PR8 virus and observed at 7 d post-infection. PE-conjugated anti-mouse Ly-6G antibody (red) and fluorescent dextran (white) were intravenously administered to visualize the vascular neutrophils and lung architectures, respectively. Green indicates virus-infected cells. Blue indicates both infiltrating (arrowheads) and vascular neutrophils (arrow). **c**, *Ly6g*$^{Cre/+}$;*R26*$^{tdTomato/+}$;*Cx3cr1*$^{GFP/+}$ mice were intranasally infected with 10^5 PFU of MA-Venus-PR8 virus and observed at 5 d post-infection. Fluorescent dextran (white) was intravenously administered to visualize the lung architecture. Red, green, and blue indicate neutrophils, monocytes, and virus-infected cells, respectively. The yellow arrowhead and arrow indicate a neutrophil and a monocyte, respectively, in contact. AB, antibody.

Influenza virus–infected lungs are infiltrated by numerous immune cells, including neutrophils and monocytes[43–45]. An immune cell–specific reporter mouse line can be used to visualize cells infiltrating the alveoli and cells in blood vessels, whereas it is preferable to label intravascular cells by intravenous administration of fluorochrome-conjugated antibodies[5,46,47]. Consistent with previous reports, intravenously injected antibodies will label only the cells in contact with the blood flow and not those in extravascular regions under our experimental conditions[5]. By administering a fluorescently labeled antibody against neutrophils into neutrophil reporter mice, we can observe the behavior of both the neutrophils infiltrating the influenza-infected lungs and the neutrophils in blood vessels separately (Fig. 5b). To observe the interaction between different kinds of infiltrating immune

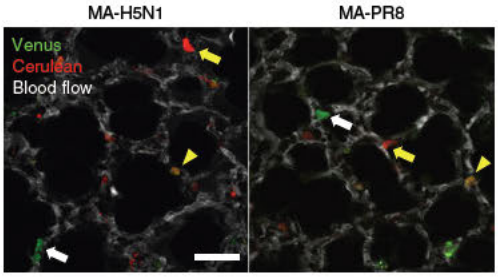

Fig. 6 | Co-infection imaging of influenza virus-infected lungs. B6 mice were intranasally infected with 10^5 PFU of MA-Venus-H5N1 and MA-Cerulean-H5N1, or MA-Venus-PR8 and MA-Cerulean-PR8 viruses and observed at 3 d (H5N1) or 4 d (PR8) post-infection. Fluorescent dextran (white) was intravenously administered to visualize the lung architecture. Red and green indicate MA-Cerulean-virus-infected cells (yellow arrows) and MA-Venus-virus-infected cells (white arrows), respectively. The yellow arrowheads indicate cells co-infected with MA-Cerulean-virus and MA-Venus-virus. Scale bar, 50 μm.

cells, such as neutrophils and monocytes, double-reporter mice expressing fluorescent proteins with different spectra but similar brightness have a major advantage (Fig. 5c and Supplementary Video 3).

Co-infection of the host with different strains of influenza virus can lead to the emergence of reassortant viruses. By infecting mice with Color-flu viruses that produce different fluorescence spectra, we detected alveolar epithelial cells that simultaneously expressed two fluorescent proteins in vivo (Fig. 6). Visualization of co-infected cells might enable us to better understand the reassortment process of influenza viruses in vivo.

In summary, the use of this in vivo imaging system for infected animal and multicolor imaging enables us to analyze pathology and immune cell dynamics at the cellular level, which would not be possible by using conventional histopathology methods. This imaging system thus provides a novel and useful approach for investigating viral pathogenicity.

Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support this study are available from the corresponding author upon reasonable request.

Code availability

The MATLAB scripts are available at https://github.com/KawaokaLab/Ueki_PNAS_2018.

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Author contributions

H.U., D.Z., and Y.K. designed the method and performed the experiments. M.G. provided a mouse line. H.U., I.-H.W., and Y.K. wrote the manuscript.

Competing interests

Y.K. is a founder of FluGen and has received speaker's honoraria from Toyama Chemical and Astellas and grant support from Chugai Pharmaceuticals, Daiichi Sankyo Pharmaceutical, Toyama Chemical, Tauns Laboratories, Otsuka Pharmaceutical, and Kyoritsu Seiyaku.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41596-019-0275-y.

Correspondence and requests for materials should be addressed to Y.K.

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Related links

Key references using this protocol

Fukuyama, S. et al. *Nat. Commun.* **6**, 6600 (2015): https://doi.org/10.1038/ncomms7600
Ueki, H. et al. *Proc. Natl Acad. Sci. USA* **115**, E6622-E6629 (2018): https://doi.org/10.1073/pnas.1806265115

nature research

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☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	To efficiently excite multiple fluorescent proteins and fluorescent dyes simultaneously, the wavelength of the infrared laser was set at 910 nm. All fluorescent spectra between 410 and 695 nm wavelengths were detected using a 20x water immersion lens (Carl Zeiss AG, Germany) and the signals were recorded in lambda image stacks.
Data analysis	We use the linear unmixing module of ZEN software for separating spectrum data. Unmixed time series stacks are subjected to image registration to correct for tissue drifts and respiratory artefacts. A reference channel is required for determining the shift and distortion of the objects. In our studies, we employ time series stacks of blood vessels or collagens for such use, as their localizations are constant over time without significant changes in shapes or structures during the observation.

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- A description of any restrictions on data availability

The data that support this study are available from the corresponding author upon reasonable request.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Only one sample was shown as a representative example that can be obtained by using the imaging protocol.
Data exclusions	No data was excluded since one representative image was shown.
Replication	No repeated measurements were performed in this paper since one image has been shown as a representative image by using the imaging protocol.
Randomization	No randomization is included in this paper since one image has been shown as a representative image by using the imaging protocol.
Blinding	Blinding was not relevant to this study which is describing a imaging protocol and anticipated results.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP seq
☒	☐ Flow cytometry
☒	☐ MRI based neuroimaging

Antibodies

Antibodies used	FITC conjugated anti mouse Ly 6G antibody (BioLegend Cat# 127606, RRID:AB_1236494). Alexa FluorR 488 conjugated anti mouse Ly 6G antibody (BioLegend Cat# 127626, RRID:AB_2561340). DyLightR 488 conjugated anti mouse Ly 6G antibody (Leinco Technologies, Cat# L287, RRID:AB_2810281). PE conjugated anti mouse Ly 6G antibody (BD Biosciences Cat# 551461, RRID:AB_394208). Alexa FluorR 594 conjugated anti mouse Ly 6G antibody (BioLegend Cat# 127636, RRID:AB_2563207). Alexa FluorR 647 conjugated anti mouse Ly 6G antibody (BBioLegend Cat# 127610, RRID:AB_1134159).
Validation	All antibodies used are commercialized and the fluorescence has been tested in this study. The Information is included in Table 4.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Madin Darby canine kidney (MDCK) cells.
Authentication	None of the cell lines used have been authenticated.
Mycoplasma contamination	All used cell stocks tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Six ten week old C57BL/6 mice (Japan SLC, Inc.) and transgenic mouse lines were used in this study. All animal care and experiments conformed to the guidelines for animal experiments of the University of Tokyo, and were approved by the animal research committee of the University of Tokyo (PA17 31 and PA17 17). All in vivo imaging studies were performed in the biosafety level 3 facility at the University of Tokyo (Tokyo, Japan), which is approved for such use by the Ministry of Agriculture, Forestry, and Fisheries of Japan.
Wild animals	Not applicable.
Field-collected samples	Not applicable.
Ethics oversight	All experiments with mice were performed in accordance with the University of Tokyo's Regulations for Animal Care and Use and were approved by the Animal Experiment Committee of the Institute of Medical Science, the University of Tokyo.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] Monthly Technical Progress Jan 2020 Report Update (Thursday, March 12, 2020)
Date: Thursday, March 12, 2020 9:38:55 AM
Attachments: image001.png
image002.png
image003.png

Thanks [redacted]
That is very usefull.
Please keep us updated on the status of the preclinical work on CoV 19.
[redacted]

From: [redacted]
Sent: Thursday, March 12, 2020 10:26 AM
To: [redacted] >
Cc: [redacted]
Subject: Re [redacted] - Monthly Technical Progress Jan 2020 Report Update (Thursday, March 12, 2020)

I am sorry for dropping out, due to an emergency meeting.

FY[redacted] has successfully completed a macaque study for SARS-VoV-2 and ferret work is ongoing. The virus replicates in macaques and causes lung lesions (intermediate to those seen with SARS and MERS CoVs), so this should be a useful model for drugs/vaccines.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]

CC: [redacted] >

[redacted] - Monthly Technical Progress Jan 2020 Report Update (Thursday, March 12, 2020)

20200311: added monthly report and supporting files from Madison; updated Webex details. Note, US started daylight savings while Europe has not. Given the rapidly evolving public health situation, we will need to discuss a virtual option for the April 24 face-to-face meeting
20200127: Added a calendar hold for monthly teleconference

HHS group calendar invite for [redacted] contract monthly TC on Thursday, March 12, 2020 (Usually second Thursday of the month)
In Person (At [redacted] – reserved 8:15am-10:30am Eastern)
Time: (Note, the differences in time since DST started in US, but has not started in Europe)
07:30 [redacted] (Daylight savings time ended Nov 3, 2019 back 1 hour; DST starts Mar 8, 2020, forward 1 hour)
08:30 [redacted] (Daylight savings time ended Nov 3, 2019 back 1 hour; DST starts Mar 8, 2020, forward 1 hour)
12:30 [redacted] (Daylight savings time ended Oct 27, 2019 back 1 hour; DST starts Mar 29, 2020, forward 1 hour)
13:30 [redacted] (Daylight savings time ended Oct 27, 2019 back 1 hour; DST starts Mar 29, 2020, forward 1 hour)
21:30 [redacted] (Thursday) (Japan: No Daylight Savings Time in 2019, 2020)
23:30 [redacted] (Friday just after midnight) (Daylight savings started Oct 6, 2019 forward 1 hour; DST ends Apr 5, 2020, back 1 hour)
Duration 1hr 30 min
Webinar and teleconference details:

Access Information:
Where: WebEx Online
To join WebEx Session, click on link: When it's time, join the meeting or https://cpe.webex.com/cpe/j.php?
[redacted]

Meeting number (access code): [redacted]
Meeting password: [redacted]

Audio Connection:

[redacted] Call-in toll number (UK)
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Meeting File:

February 2020 Monthly Report (Year 5, Report 4) –
– covering both the [redacted] Email
from 11 March 2020 (Due Friday, 6 March 2020)
Reporting Period: February 2020
Report submitted: Wednesday, 11 March 2020
[redacted] Feb 23, 2020)

HI Assay – February 2020

Minutes from last month's [redacted] teleconference on Thursday, February 13, 2020
Email from XXX, XX, 20XX

From: [redacted]
To: [redacted]
Subject: RE: Virtual site visit
Date: Monday, April 6, 2020 3:19:00 PM

Dear All,

I think it is very difficult to get people to focus on anything at this point with a normal mindset. However, the major goal of this call should be future funding. To that end, we should calculate back what we want to present. In other words, let's list the areas for which we need funding for the next 5 years, present the data/interpretation on those topics, and identify what needs to be done.

At the end of the above presentations, [redacted] could briefly summarize the presentations and lead the discussion on future funding. We should leave one hour for the discussion on future funding.

Since we know how these discussions go (i.e., a lot of questions), our presentations should be high level (while avoiding the details) and we should make sure we finish this part in 3.5 hours including a break.

I believe I am saying the same thing as [redacted] and [redacted]. But, let's start by identifying the topics for which we want funding and making an agenda for the call.

Best,
[redacted]

From: [redacted]
Sent: Monday, April 6, 2020 11:52 PM
To: [redacted]
Subject: RE: Virtual site visit

Dear All,

I am wondering if we should focus more on conceptual issues, and go light on experimental data.
This would also raise the question if there should be multiple presentations from multiple

people (which can be a bit confusing and/or disjointed at times), or if we should have a small number of main presentations to which several people contribute slides.
I am flexible and open to suggestions – but with unpredictable schedules and ~ 2 weeks left, we may want to start thinking about this.

Thanks,

[redacted]

From: [redacted]
Sent: Monday, April 6, 2020 9:35 AM
To: [redacted]
Cc: [redacted]
Subject: Re: Virtual site visit

We have 4.5h, [redacted] said this would be adjusted based on the agenda.

For sure [redacted] will want to hear about [redacted] status.

We should also make sure our agenda is in there too, which is primarily about the next funding. So what we plan to do next on the [redacted] front. And status and future on the [redacted] too. So, showing our status for [redacted] will be important, the stuff we are doing to show this as titers, as well as maps, is directly related to that. I think we should probably go light on the [redacted], just saying there is background work on this, rather than a detailed explanation--you and [redacted] suggested that for the last site visit and I think it makes sense, do you agree?

Also taking them through the recent VCM choices, especially the +40% increase in VE by the [redacted], and being hooked up with [redacted] on that, and what can be done next. To that end, I emailed [redacted] for a meeting on this topic on March 11. Got an enthusiastic reply, but no action. Figured he got sucked into the CoV vortex, which he confirmed today, and he will try to find time to call.

What do you think?

[redacted]

On Mon, Apr 6, 2020 at 11:29 AM [redacted] > wrote:

> Dear All,
>
> For the virtual site visit, shall we all prepare presentations at usual?

Or has [redacted] indicated that we should focus on one topic (I guess that would be [redacted]), and touch on other topics only briefly? I believe we have only half a day.

Thanks,

[redacted]

From: [redacted]
Sent: Monday, April 6, 2020 12:45 AM
To: [redacted]
Subject: No [redacted] call today

No [redacted] call today. We'll schedule one closer to the [redacted] virtual site visit.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: A phone call to discuss future collaboration
Date: Thursday, March 12, 2020 7:22:44 AM

Noted thanks [redacted]

[redacted]

-------- Original message --------

From: [redacted]

Date: Thu, 12 Mar 2020, 12:20

To: [redacted]

Cc: [redacted]

Subject: RE: A phone call to discuss future collaboration

[redacted]et al.

I am at a conf call of Expert Meeting on Novel Coronavirus Disease Control in Japan.

I will join as soon as possible.

[redacted]

From: [redacted]
Sent: Thursday, March 12, 2020 9:10 PM
To: [redacted]
Cc: [redacted]
Subject: Fwd: A phone call to discuss future collaboration

[redacted] seems positive, see below. If you have any particular thoughts prior to me talking to him, please let me know.

[redacted]

---------- Forwarded message ---------
From: [redacted]
Date: Wed, Mar 11, 2020 at 7:37 PM
Subject: RE: A phone call to discuss future collaboration
To: [redacted]

Yes I think would be excellent. Friday?

From: [redacted]
Sent: Wednesday, March 11, 2020 2:54 PM
To: [redacted]
Subject: A phone call to discuss future collaboration

[redacted]

Would you be up for a phone call to discuss moving forward together on a bunch of [redacted] flu stuff? It seems like it could be great.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] work, contract activities during shutdown
Date: Thursday, April 2, 2020 6:08:17 AM
Attachments: image001.png
image002.png
image003.png

Yes, good changes, agree.

[redacted]

On Thu, Apr 2, 2020 at 12:05 PM [redacted] wrote:

> Based on [redacted] reply, should we change it to the following statements?
>
> *>>but adjusted because of the* [redacted] *in* [redacted]
>
> Change to: "but adjusted because of reduced experimental work in [redacted]"
>
> *>>The* [redacted] *is delaying our work toward* [redacted]
>
> Change to: "Reduced experimental work in [redacted] is delaying our work toward [redacted]
>
> [redacted]
>
> ---
>
> **From:** [redacted]
> **Sent:** Thursday, April 2, 2020 5:55 AM
> **To:** [redacted]
> **Cc:** [redacted]
> **Subject:** Re [redacted] work, contract activities during shutdown
>
> Agreed. I think it is safer to use the same wording for [redacted]. We may also do some work that can not be postponed.
>
> [redacted]
>
> Yours sincerely,
>
> [redacted]

[redacted]

[redacted]

CC: [redacted]

Onderwerp: RE: [redacted] work, contract activities during shutdown

Dear All,

We would suggest the following changes:

>>but adjusted because of the [redacted] *in* [redacted]

Change to: "but adjusted because of the [redacted] and reduced [redacted]

>>The [redacted] *is delaying our work toward* [redacted]

Change to: "The [redacted] and [redacted] are delaying our work toward [redacted]

Best,

[redacted]

From: [redacted]
Sent: Thursday, April 2, 2020 2:48 AM
To: [redacted]

[redacted]
Cc: [redacted]
Subject: Re[redacted]work, contract activities during shutdown

Hi all,

Our proposed reply to [redacted]

The work of the [redacted] *on* [redacted] *continues, but adjusted because of the* [redacted]

Work in [redacted] *is unaffected, other than working from home. Work in* [redacted] *is unaffected. In* [redacted] *and* [redacted] *staff are working primarily on analyses, also on experimental design for when* [redacted] *Virtual lab meetings, and meetings among our laboratories continues.*

The [redacted] *is delaying our work toward* [redacted]

No funds from these options is being used on Covid-19 work.

Please let us know if you you think we need to change anything.

Thanks,

[redacted]

On 29 Mar 2020, at 13:46, [redacted] wrote:

[redacted]

Thank you for sharing the information!

[redacted]

-----Original Message-----
From: [redacted]
Sent: Sunday, March 29, 2020 9:39 PM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]work, contract activities during shutdown

Hi [redacted]
I already responded about my [redacted] base contract (see below) because I think that [redacted] would coordinate a response for the [redacted] only, and it would be unlogical if he would also write about our base contracts and options on completely different topics.
Kind regards
[redacted]

[redacted]
Our labs have completely shut down for non-essential work, but we continue essential work including work on coronavirus. In practice, this means that our [redacted] work is still somewhat continuing, in particular because of the ongoing detections of [redacted] Our PhD students, post-docs, PIs are mostly working on data analyses and manuscript writing. In particular the folks that are on the Options.

Some of the technical personnel and animal experimentalists have been partly shifted to assist in the diagnostics (e.g. setting up new methods now that there is a shortage on diagnostic reagents), NGS (implementing real-time minion NGS, similar to what we developed with [redacted]), to macaque experiments to study pathogenesis, to study virus transmission in ferrets and to measure virus in aerosols and droplets. This is all done with personnel on the base contract.

Kind regards,

Yours sincerely,
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

How are you going to respond to the request, considering that [redacted] cannot be re-directed? As [redacted] suggested, we may want to coordinate our response.

Best,

[redacted]

-----Original Message-----
From: [redacted]
Sent: Friday, March 27, 2020 5:09 AM
To: [redacted]
Cc: [redacted]
Subject [redacted] work, contract activities during shutdown

Hi all,

For [redacted] questions re contract activities during the shutdown, [redacted] would like us please to coordinate on our replies to [redacted] for our [redacted] options. This will allow us to better coordinate with [redacted] re [redacted] activities. Let's discuss in our call today if we have time. The questions are below FYI:

- Are you working in the lab on flu right now?

- If you are not working on flu what [redacted] elated activities are you doing at remotely (examples writing papers, data analysis, lab meetings)?

- Are you working on COVID using [redacted] funds? If yes have you cleared this with [redacted] ? How will this impact your flu studies?

Many thanks and hope you are all well,

[redacted].

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]
Date: Sunday, March 1, 2020 2:04:01 AM

Thanks to you and your team, [redacted], for all the hard work!
[redacted]

From: [redacted]
Sent: Saturday, February 29, 2020 8:43 PM
To: [redacted]
Cc: [redacted]
Subject: [redacted]

Dear all,

Our [redacted] proposal was just submitted. Thanks to everybody for their big effort in getting this done. I have included a copy of the technical proposal minus the finances.

Good luck to all of us!

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] Monthly Technical Progress Jan 2020 Report Update (Thursday, March 12, 2020)
Date: Thursday, March 12, 2020 9:25:53 AM
Attachments: image001.png
image002.png
image003.png

I am sorry for dropping out, due to an emergency meeting.

FYI: [redacted] has successfully completed a macaque study for SARS-VoV-2 and ferret work is ongoing. The virus replicates in macaques and causes lung lesions (intermediate to those seen with SARS and MERS CoVs), so this should be a useful model for drugs/vaccines.

[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted]

CC: [redacted]

[redacted] - Monthly Technical Progress Jan 2020 Report Update (Thursday, March 12, 2020)

20200311: added monthly report and supporting files from Madison; updated Webex details. Note, US started daylight savings while Europe has not. Given the rapidly evolving public health situation, we will need to discuss a virtual option for the April 24 face-to-face meeting
20200127: Added a calendar hold for monthly teleconference

[redacted] contract monthly TC on Thursday, March 12, 2020 (Usually second Thursday of the month)
In Person (At [redacted] reserved 8:15am-10:30am Eastern)
Time: (Note, the differences in time since DST started in US, but has not started in Europe)
07:30 Madison (Daylight savings time ended Nov 3, 2019 back 1 hour; DST starts Mar 8, 2020, forward 1 hour)
08:30 [redacted] (Daylight savings time ended Nov 3, 2019 back 1 hour; DST starts Mar 8, 2020, forward 1 hour)
12:30 [redacted] Daylight savings time ended Oct 27, 2019 back 1 hour; DST starts Mar 29, 2020, forward 1 hour)
13:30 [redacted] (Daylight savings time ended Oct 27, 2019 back 1 hour; DST starts Mar 29, 2020, forward 1 hour)
21:30 [redacted] Thursday) (Japan: No Daylight Savings Time in 2019, 2020)
23:30 [redacted] (Friday just after midnight) (Daylight savings started Oct 6, 2019 forward 1 hour; DST ends Apr 5, 2020, back 1 hour)
Duration 1hr 30 min
Webinar and teleconference details:
Access Information:
Where: WebEx Online
To join WebEx Session, click on link: When it's time, join the meeting or https://cpe.webex.com/cpe/j.php?
[redacted]
Meeting number (access code) [redacted]
Meeting password: [redacted]
Audio Connection:
[redacted] Call-in toll number (UK)
[redacted] Call-in toll-free number (UK)
[redacted]
Need more numbers or information? Global call-in numbers | Toll-free calling restrictions.|
Meeting File:
February 2020 Monthly Report (Year 5, Report 4) –
– covering both the [redacted] - Email
from 11 March 2020 (Due Friday, 6 March 2020)
Reporting Period: February 2020
Report submitted: Wednesday, 11 March 2020
[redacted] (Feb 23, 2020)
HI Assay – February 2020
Minutes from last month's [redacted] teleconference on Thursday, February 13, 2020
Email from XXX, XX, 20XX

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: FW: Research Project 2
Date: Sunday, February 16, 2020 7:40:00 AM
Attachments: pages 124-136 Section 4A.4 Res Proj 2 2-15-2020 - [redacted] docx

---

[redacted]

Please see attached. Just a few minor changes.

Thank you for your hard work!

[redacted]

From: [redacted]>
Sent: Sunday, February 16, 2020 1:21 PM
To: [redacted]
Cc: [redacted]>
Subject: Research Project 2

Hi,

I basically did not change anything here, it looks good. In any case, have a look to see whether you find something you want to change

[redacted]

From: [redacted]
To: [redacted]
Subject: [redacted]
Date: Thursday, February 13, 2020 12:15:00 PM
Attachments: [redacted].docx

Dear all,

Please see attached data that I was talking about during our internal call.
We are doing this experiment to mount immunity to establish a preimmune animal model, the exact topic that was discussed during the [redacted] call just now.
We do not want to use live virus to mount the immunity because I want to use this animal model to test an [redacted]; if we use live virus for mounting immunity, the immunity to NP will provide protection upon challenge.

The data attached are self-explanatory. We are testing different adjuvants and also immunizing animals with [redacted] in nanoparticle form.

Best,
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Alternative dates for [redacted] site visit
Date: Tuesday, January 28, 2020 7:58:00 PM

[redacted]

Have we decided the dates?
My days on April are filling.

[redacted]

From: [redacted]
Sent: Friday, January 10, 2020 7:08 PM
To: [redacted]
Cc: [redacted]
[redacted]>
Subject: Re: Alternative dates for [redacted] site visit

Apologies, minor amendment to the dates in Option 1. Please confirm re site visit.

Option 1.
- internal meeting on Thursday March 12th
- main site meeting with [redacted] on Friday March 13th

Option 2.
- internal meeting on Tuesday March 31st
- main site meeting with [redacted] on Wednesday April 1st

Option 3.
- internal meeting on Wednesday April 1st
- main site meeting with [redacted] on Thursday April 2nd

[redacted]

On 10 Jan 2020, at 10:04, [redacted] > wrote:

Dear all,

[redacted] and I have been exploring alternative dates for our intended site visit.

We have identified the below dates which we think should work given the information we have received to date. We cannot have a site visit too near the beginning of March in case the [redacted] data does not come to us until end of Feb.

Could you please confirm that the options below would indeed work for you? We will then propose these as new alternatives to [redacted] et al.

Option 1.
- internal meeting on Thursday March 11th
- main site meeting with [redacted] on Friday March 12th

Option 2.
- internal meeting on Tuesday March 31st
- main site meeting with [redacted] on Wednesday April 1st

Option 3.
- internal meeting on Wednesday April 1st
- main site meeting with [redacted] on Thursday April 2nd

Many thanks

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted] & coronavirus
Date: Wednesday, January 29, 2020 1:48:00 PM

Dear [redacted] and [redacted]

Here is what my labs in Madison and Tokyo are going to do on 2019-nCoV:

1. Examine the pathogenesis of 2019-nCoV in different animals including marmosets, macaques, dogs, cats, ferrets, hamsters, and mice

2. Establish a point-of-care rapid diagnostic kit; there are 26 companies in Japan who make these kits for influenza. I collaborated with one of them on an H7 detection kit and will do the same for this virus

3. Develop an mRNA vaccine with a Japanese company

4. Study host responses in patients infected with 2019-nCoV

[redacted]

From: [redacted]
Sent: Thursday, January 30, 2020 4:22 AM
To: [redacted]
Cc: [redacted]
Subject: [redacted] & coronavirus

Dear all,

I got a call from [redacted] this afternoon relating to the coronavirus and potential impacts upon our project. Some points from the call as follows (some more pertinent to [redacted] but included for reference):

1. [redacted] staff are being switched to coronavirus related matters as a priority.

2. Therefore if we have any urgent matters for [redacted] we need to get them over ASAP. If you have any please let myself and [redacted] know.

3. This may further delay the [redacted] study data. In particular leadership are unlikely to be able to really review into the [redacted] study. A likely outcome is that [redacted] give us their take and then we further peer review the data in our own time, but perhaps not before the site meeting. In short [redacted] study remains a somewhat unknown quantity at present.

4. [redacted] asked that if we have any knowledge, involvement or anything related to coronavirus, could we share it in the spirit of collaboration. I believe [redacted] are reaching out to all their scientific partners in this manner. [redacted] do not have anything, but <u>if you could confirm for your labs that would be helpful thanks.</u>

5. [redacted] has been assigned to coronavirus analytics, there is a very small chance [redacted] may ask for some help with these analytics - to what extent and in what capacity remains unknown at present.

6. In general, all [redacted] staff will be less available than usual. I have indicated to [redacted] that of there is anything project related he wishes to push towards us we will be happy to help lighten their workload wherever possible.

7. I also updated [redacted] again that we are actively pursuing the budget and timelines for CVV production, with intention to get proposal to [redacted] t earliest possible opportunity.

Many thanks

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted]
Date: Saturday, February 29, 2020 1:55:00 PM

Thanks, [redacted]!

[redacted]

From: [redacted]
Sent: Sunday, March 1, 2020 4:43 AM
To: [redacted]
Cc: [redacted]>
Subject: [redacted]

Dear all,

Our [redacted] proposal was just submitted. Thanks to everybody for their big effort in getting this done. I have included a copy of the technical proposal minus the finances.

Good luck to all of us!

[redacted]

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Subject: RE: FW: [redacted] website
Date: Monday, February 10, 2020 10:52:00 AM
Attachments: image001.png
image002.png
image003.png

Dear [redacted]

Thank you for letting me know. I have decided not to spend any more of my time discussing this topic with reporters. Instead, I have been asking [redacted], who is the RO of our select agent program and [redacted] who is our communications person, to respond to reporters.
If the reporter wants to speak with [redacted], please let me know. I will connect him to them.

Best,
[redacted]

From: [redacted]
Sent: Tuesday, February 11, 2020 1:23 AM
To: [redacted]
Subject: FW: FW: [redacted] website

> Hi [redacted]
> This person has approached you before for an interview. I have talked to him about GOF research, and he seemed to understand it and appreciate the work we do. Also the news outlet he works with seems to do reasonably balanced stories in general. Although I certainly do not want to put any pressure on you, I said to him I would share my personal opinion about the interview with you, so you might (re)consider talking to him as well.
> Kind regards
> [redacted]
>
> Yours sincerely,
>
> [redacted]

[redacted]

From: [redacted]
Sent: Monday, February 10, 2020 4:31 PM
To: [redacted]
Subject: Re: FW: [redacted] website

Hello [redacted]!

I was following up on if you'd be comfortable asking [redacted] to reconsider speaking with me? I'd especially like to go to his lab and go through all the safety procedures, to highlight exactly what these labs look like.

Thank you!

—

On Mon, Jan 27, 2020 at 10:38 AM wrote:

Perfect! I'll get in touch with .

—

On Mon, Jan 27, 2020 at 10:22 AM > wrote:

Yes, that is OK

Yours sincerely,

From: >
Sent: Monday, January 27, 2020 5:03 PM
To:
Subject: Re: FW: website

Thanks,

Editorial standards make it so I cannot send you the *actual* words in an article. What I do is send a fact-checking email which paraphrases what I am going to say to ensure it is factually correct. Is that acceptable to you? I understand the sensitivity and sensationalization that comes with this topic.

—

On Mon, Jan 27, 2020 at 8:11 AM > wrote:

Dear

I can make myself available for an interview on this topic. The only request I have for interviews like this is that I will be offered the opportunity to correct your article for factual errors and erroneous quotes and quoting me out-of-context.

If that is acceptable, you can schedule an appointment with [redacted] copied).
Kind regards,
[redacted]

Yours sincerely,

[redacted]

[redacted]

Begin forwarded message:

From: [redacted] >
Subject: [redacted] **website**
Date: 23 January 2020 at 18:32:32 CET
To: [redacted]

[redacted]
Naam: Zarley
Email adres: b.david@freethink.com
Pagina titel: [redacted]
Pagina adres: [redacted]
Vraag of opmerking:
[redacted] My name is B. David Zarley. I'm a Chicago-based senior staff writer for Freethink, and I'm looking to write an article about gain of function experiments. It won't look to adjudicate whether they are necessary, merely present both sides without a preference. Would you be willing to be interviewed for this article? Thank you!

From: [redacted]
To: [redacted]
Subject: RE: [redacted]
Date: Thursday, February 13, 2020 12:57:53 PM

Dear All,

Tomorrow, I am available all day except from 9:30-10:30 am Central Time for the CEIRS seminar series.

[redacted]

From: [redacted]
Sent: Thursday, February 13, 2020 9:56 AM
To: [redacted]
Subject: RE: [redacted]

I am available:

Tomorrow: 6:30am-7:30am (I will be in a car); after 10am

I will not be available on Monday; I will be on a flight to Japan.

[redacted]

From: [redacted]
Sent: Friday, February 14, 2020 12:34 AM
To: [redacted]
Subject: Re: [redacted]

Hi everyone,

Could you please inform me of your availability tomorrow and Monday for an hour discussion, continuing the 30 mins discussion prior to the [redacted] call today?

Many thanks

[redacted]

On 13 Feb 2020, at 12:53, [redacted]> wrote:

Dear all,

Please find attached preliminary data from additional titrations we performed. We titrated the [redacted] from Madison against the sera in our [redacted] map. The sera are labeled on the x axis.

With kind regards,

[redacted]

From: [redacted]
Date: Thursday, 13 February 2020 at 12:46
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]

Thanks everyone. We're about to send out a webex invite for a call 30 minutes before the [redacted] call today.

[redacted]

On Tue, Feb 11, 2020 at 11:26 PM [redacted]> wrote:

> I can talk before and after the [redacted] call.
>
> [redacted]
>
> ---
>
> **From:** [redacted]
> **Sent:** Wednesday, February 12, 2020 8:12 AM
> **To:** [redacted]
> **Cc:** [redacted]
> **Subject:** RE: [redacted]
>
> I can talk before the [redacted] call as well.
>
> Thanks,
>
> [redacted]

From: [redacted]
Sent: Tuesday, February 11, 2020 10:53 AM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]

I can start 30 min before the call, but I have another meeting after the [redacted] call.

From: [redacted]
Date: Tuesday, 11 February 2020 at 17:52
To: '[redacted]>
Cc: [redacted]
Subject: Re: [redacted]

I can stay on the phone after the [redacted] call or start half an hour before the [redacted] call.
[redacted]

[redacted]>
CC: [redacted]
[redacted]**:** Re: [redacted]

Many thanks [redacted] Also for the clear text explanation and very helpfully marked and laid out excel sheets.

In addition to what you say, the [redacted] (but not [redacted]), does "reach a little further into the WT portion of the map, or at least has some low titers against a few strains the other sera do not see.

interesting indeed, as you point out, that the [redacted]. even if we don't see the pattern of general increased immunogenicity that was seen for the [redacted] candidate, this could be a reason to go with a [redacted] as maybe the reason for this result is that

antibodies targeting the [redacted].

seems to me our path of try [redacted]
and some of the [redacted] is still a good thing to do.

For me would be good to have a call to discuss these results and our path forward. Could it work for others to do this by staying on the WebEx after the [redacted] call on Thursday. Or to have a call before the [redacted] call on Thursday?

[redacted]

On Tue, Feb 11, 2020 at 11:43 AM [redacted] > wrote:

> Dear all
> Please find attached the results of an HI that was done to test the [redacted]
> - [redacted]
> - [redacted]
> - [redacted]
> - [redacted].
>
> Happy to discuss over the phone should it be necessary.
> Cheers
> [redacted]

From: '[redacted]
Date: Friday, 31 January 2020 at 18:01
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]

Thanks [redacted]

[redacted]

On Fri, Jan 31, 2020 at 4:17 PM [redacted] > wrote:

> There are [redacted]
>
> Link to a pc of the combined maps (without [redacted]
>
> [redacted]
>
> On 2020-01-31 08:51, [redacted] wrote:

> Thanks [redacted] for your post call notes. I agree re you sending your
> [redacted]. Some other suggestions:
>
> - I have not looked to see if [redacted] is included in that set, but
> would be good to send it, along with say [redacted].
> This is because resolving the relative position of [redacted] is
> especially important for our vaccine study design.
>
> - Similarly would be good to include sera from the infection and
> different vaccine formulations of the [redacted] (I think you were
> including these anyhow).
>
> - Include the [redacted] This is to help resolve any differences between
> those strains between the two maps, and so there are not having to be
> two of those strains in the merged map if it can be avoided. I've
> copied [redacted] for the list of those common strains.
>
> [redacted] In addition to the viruses you suggest to send to [redacted],
> would be good also to send:
>
> - your four, I think four, [redacted]. This for the same reason I wrote to Mathilde above.
>
> - The [redacted] (as also
> suggested for [redacted])
>
> - And also a selection of your other reference WT viruses and sera.
> You have done lots of valuable and repeated titrations of those and
> ideally we have a robust integration of the two maps, not just of the
> [redacted].
>
> - Please also include the [redacted] for
> coordination.
>
> [redacted]
>
> On Fri, Jan 31, 2020 at 12:49 AM [redacted] wrote:
>
>> Hi [redacted]
>>
>> Thanks for the sequence and other information!
>>
>> At this point, the [redacted] may be the best
>> viruses to send your way.
>>
>> With additional HI data and the (re)creation of mutants with [redacted] some of these viruses could be sent as
>> well.

>>
>> Thanks,
>>
>> [redacted]
>>
>> From: [redacted]
>> Sent: Wednesday, January 29, 2020 10:07 AM
>> To: G[redacted]
>> Cc: [redacted]
>> Subject: Re: [redacted]
>>
>> Dear all
>>
>> A quick follow up email on some of the points that we have discussed
>> today:
>>
>> *[redacted], please find attached the sequence of our [redacted]
>> [redacted]
>> [redacted]
>> [redacted]
>> * [redacted], please find here two links to [redacted] website
>> explaining the generation of the [redacted] and
>> what we call the [redacted]). In both cases, we
>> have compared the maps to the full map and, they were representative
>> of the full map. [redacted], you will not be able to look at the
>> webpage I think, if I you want I can walk you through them another
>> time [redacted], I remember that [redacted] presented that data one time
>> during a [redacted] meeting that you attended via Webex, at the time
>> that we were discussing exchanging strains and sera for map
>> comparison).
>>
>>
> [redacted]
>>
>>
>>
> [redacted]
>>
>>
>> * My suggestion is to send the strains and sera from the [redacted]
>> [redacted] When we do so, we can also send the [redacted]
>> [redacted]. We could share our [redacted]
>> [redacted]. We also have our six

>> recent post-vaccination sera raised with [redacted]
>> [redacted]. However, we have very limited amount of these
>> (they were taken a week before challenge from a draw of about 5ml of
>> blood). We are also gonna titrate the post challenge sera. Should
>> these be similar to the pre-challenge sera, we will be able to share
>> them easily. We should have the data soon.
>> * Next week, we will bleed the ferrets for sera production
>> [redacted] Please
>> remember as I mentioned today that we were not able to have a
>> [redacted] (sorry, I did
>> not realize that [redacted]
>> [redacted]). We
>> will titrate these sera against at least the [redacted] and the
>> [redacted] (we are titrating our vaccine sera too). Moreover,
>> we will titrate the corresponding viruses against sera of the [redacted]
>> and against our latest vaccine sera. From these titrations, we will
>> have a preliminary view as to whether [redacted]
>> [redacted]
>> [redacted]
>> [redacted]
>> * Just some additional information about the cross-reactivity of
>> [redacted]. Titers of [redacted] virus against
>> [redacted]
>> [redacted]
>> [redacted]
>> [redacted]
>> [redacted]
>> [redacted]
>> [redacted]
>> [redacted]
>>
>> All the best
>>
>> [redacted]
>>
>> From: [redacted]
>> Date: Saturday, 25 January 2020 at 22:59
>> To: "[redacted]
>> Subject: RE: [redacted]
>>
>> Dear All,
>>
>> Attached please find our estimated timelines for the further
>> characterization of the current vaccine candidates, and for the
>> generation and characterization of additional vaccine candidates. I

>> realize that the attached information is not easy to digest –
>> please let me know if you would like to set up another call to
>> discuss this further.
>>
>> In a nutshell,
>>
>> - the current [redacted]
>> [redacted]) will be tested in Feb with
>> additional sera.
>>
>> - the current [redacted]
>> [redacted]
>> [redacted]
>> [redacted] These
>> data should be available by the end of May.
>>
>> - the viruses already [redacted] will
>> be tested in HI assays in Feb.
>>
>> - the further development of [redacted]
>> [redacted] will take too long. Theoretically, we could combine some tasks
>> (groups 5-7), but this would create too much work in parallel to
>> group 3. Hence, groups 5, 6, 7, and 5-7 are shown in gray.
>>
>> As for the HI assay in Feb (see [redacted] HI Table'), we are planning
>> to test
>>
>> - the reference viruses and sera (shown in red font),
>>
>> - the [redacted] and sera (shown in red
>> font),
>>
>> - the viruses that have been created with [redacted] already
>> (shown in blue font),
>>
>> - sera to some of the [redacted] (shown in gold
>> font). We are still debating whether these sera should be included
>> since the viruses mutate in eggs.
>>
>> If you have any changes or additions for the HI assay, please let us
>> know by Thursday so that we can plan accordingly.
>>
>> Thanks,
>>
>> [redacted]
>>
>> From: [redacted]
>> Sent: Sunday, January 19, 2020 8:36 PM
>> To: [redacted]
[redacted]

>> [redacted]
>> Subject: FW: [redacted]
>>
>> Dear All,
>>
>> Please let us know which sera we should use to further characterize
>> the [redacted].
>>
>> Obvious candidates are the homologous sera and sera to some of the
>> reference viruses. If there are other sera that should be included,
>> please let us know as soon as possible so that we can start
>> preparing for the HI assays.
>>
>> Thanks,
>>
>> [redacted]
>>
>> From: [redacted]
>> Sent: Friday, January 17, 2020 7:25 PM
>> To: [redacted]
>> Cc: [redacted]
>> Subject: FW: [redacted]
>>
>> I noticed that [redacted] has not been copied on these mails –
>> Sorry, [redacted].
>>
>> [redacted]
>>
>> From: [redacted]
>> Sent: Friday, January 17, 2020 7:13 PM
>> To: [redacted]
>> Cc: [redacted]
>> Subject: RE: [redacted]
>>
>> Dear All,
>>
>> Attached please find the following:

>>
>> - The slides presented earlier today
>>
>> - The Excel file with the HI raw data (I added explanations
>> on the first tab; please let me know if it's not clear)
>>
>> - The [redacted] data, which we received right after
>> our call. They are interesting (please see the comments on the third
>> slide).
>>
>> - Below, I added some numbers in red font (I'll work with
>> our group to calculate timelines)
>>
>> - One topic that we didn't discuss today: The switch to [redacted]
>> [redacted] (at which point would we switch to [redacted] How much retesting are
>> we going to do for viruses with [redacted] – This will affect the
>> timelines.
>>
>> Best,
>>
>> [redacted]
>>
>> From: [redacted]
>> Sent: Friday, January 17, 2020 12:22 PM
>> To: [redacted]
>> Cc: [redacted]
>> Subject: [redacted]
>>
>> Are there other categories of viruses and sera than the below for
>> which it would be idea to have HI titers? Or some of the below
>> which are not necessary?
>>
>> Some of these titrations will already have been done. And of course
>> we might not need want or have time to do them all. But figured
>> would be good to get an ideal set at least listed.
>>
>> · HI titrations of interest for these viruses and sera
>>
>> * The reference wildtypes – we've tested ~35 (but probably
>> wouldn't have to include them all)
>> * The [redacted]
>> * The [redacted] –
>> 24 + 24 = 48 viruses (for some or all of the mutants before
>> egg-passage, we should also have the homologous sera)
>> * The viruses for which [redacted] has been replaced by [redacted] (with and
>> without 2[redacted] – 6 viruses

>> * [redacted]) (wasn't a [redacted] virus, or viruses
>> already sent to [redacted] Note, imo we should not delay the HI above
>> waiting for this virus) – I'll have to double-check
>>
>> [redacted] would you circulate your slides from today please, and also
>> the excel you were showing at the end please.
>>
>> [redacted]

<200212_HI_titer_plots.pdf>

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Research Project 2
Date: Sunday, February 16, 2020 5:22:00 AM

Thanks, [redacted]
We will get back to you shortly.

[redacted]

From: [redacted]
Sent: Sunday, February 16, 2020 1:21 PM
To: [redacted]
Cc: [redacted]
Subject: Research Project 2

Hi,

I basically did not change anything here, it looks good. In any case, have a look to see whether you find something you want to change

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Research Project 2
Date: Sunday, February 16, 2020 8:21:37 AM

Thanks [redacted]

From: [redacted]
Sent: Sunday, February 16, 2020 8:42 AM
To: [redacted]
Cc: [redacted]
Subject: FW: Research Project 2

USE CAUTION: External Message.

[redacted]

Please see attached. Just a few minor changes.

Thank you for your hard work!

[redacted]

From: [redacted]
Sent: Sunday, February 16, 2020 1:21 PM
To: [redacted]
Cc: [redacted]
Subject: Research Project 2

Hi,

I basically did not change anything here, it looks good. In any case, have a look to see whether you find something you want to change

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Your [redacted] viruses
Date: Thursday, February 13, 2020 3:16:00 PM

Thanks, [redacted]

I got those viruses from you while ago.
Best,

[redacted]

From: [redacted]
Sent: Friday, February 14, 2020 6:15 AM
To: [redacted] >
Cc: [redacted]
Subject: Re: Your [redacted] viruses

Hi [redacted]
Not a problem of course. Do you have these already? Otherwise I need to check.
[redacted]

[redacted]
[redacted]
[redacted]
CC: [redacted]
[redacted]**:** Your [redacted] viruses

Dear [redacted],

As part of the NIH CIVIC program (https://www.nih.gov/news-events/news-releases/nih-forms-new-collaborative-influenza-vaccine-research-network), the NIH wants to have a list of reference viruses for serological studies. I suggested that we should use your [redacted]. For [redacted], the virus panel should include the pandemic virus, historical viruses, and a recent virus. A recent virus is not an issue, but for historical viruses including the pandemic virus, [redacted] Therefore, I want to suggest that the following viruses be

included in the panel:

[redacted]

So, my question for you is: Are you okay with sharing these viruses with the NIH [redacted] program?

Please let me know.

[redacted]

From: [redacted]
To: [redacted]
Subject: RE: cells to isolate WH-CoV
Date: Wednesday, January 22, 2020 2:46:00 PM

Thanks, [redacted]

[redacted]

From: [redacted]
Sent: Thursday, January 23, 2020 5:47 AM
To: [redacted]
Subject: Re: cells to isolate WH-CoV

We will do that. Let us know when you have the World Courier information and we'll take the next steps. Thanks!

[redacted]

From: [redacted]>
Sent: Wednesday, January 22, 2020 3:43 PM
To: [redacted]
Subject: RE: cells to isolate WH-CoV

Dear [redacted]

Please both send DBT (delayed brain tumor) cells expressing human ACE2 and those not expressing human ACE2.

Thanks,

[redacted]

From: [redacted]>
Sent: Wednesday, January 22, 2020 11:19 PM
To: [redacted]>
Cc: [redacted]>
Subject: Re: cells to isolate WH-CoV

There will also be DBT (delayed brain tumor) cells expressing human ACE2, and i'm told we

could also send non-expressing DBT cells if necessary.

Please let me know if you need any more info about these lines. Thanks!

[redacted]

From: [redacted]>
Sent: Wednesday, January 22, 2020 9:08 AM
To: [redacted]
Cc: [redacted]
Subject: Re: cells to isolate WH-CoV

[redacted]

These will be vero cells.

[redacted]

From: [redacted]
Sent: Sunday, January 19, 2020 2:42 AM
To: [redacted]
Cc: [redacted]>
Subject: Re: cells to isolate WH-CoV

Dear [redacted]

Thank you for your information.
I will make arrangement for shipping to World courier soon.

Could you tell me the name of cell line to fill in the invoice?
I check whether we need the document for clearance at import.

Best regards,
[redacted]
On 2020/01/18 1:12, [redacted] wrote:
> [redacted]
>
> You can contact me going forward regarding this shipment. Our shipping address is:
>
> [redacted]
>
> I'm going to work on getting our export clearance which we'll need before we can ship. Thanks!
>
> [redacted]

> [redacted]
>
> ----------
> *From:* [redacted]
> *Sent:* Friday, January 17, 2020 8:54 AM
> *To:* [redacted]
> *Cc:* [redacted]
> *Subject:* RE: cells to isolate WH-CoV
> Hi [redacted], I have cc'd the key people in my laboratory. They should be in touch shortly. [redacted]
>
> -----Original Message-----
> From: [redacted]
> Sent: Thursday, January 16, 2020 9:26 PM
> To: [redacted]
> Cc: [redacted]
> Subject: Re: cells to isolate WH-CoV
>
> Dear [redacted]
>
> I am [redacted] technical staff at [redacted] Tokyo lab and make arrangement of transfer of materials.
>
> I need your information to contact World courier.
> Could you let me know contact person and information for shipping?
>
> Best regards,
> [redacted]
>
> **********
> [redacted]
> **********
>
> On 2020/01/17 10:44, [redacted] wrote:
>> Dear [redacted]
>>
>> It was good to talk with you.
>>
>> I am writing here to introduce [redacted] who will coordinate getting a cell line to Japan suitable for isolating the new coronavirus. She will work with World Courier to get the cells from your lab to Tokyo.
>>
>> I very much appreciate your help.

>>
>> If we are successful in isolating the virus, everyone can have access to it.
>>
>> [redacted]
>>
>> *From:*[redacted]
>> *Sent:* Friday, January 17, 2020 9:06 AM
>> *To:*[redacted]
>> *Subject:* RE: cells to isolate WH-CoV
>>
>> Hi [redacted]. Anytime tonigh[redacted]
>>
>> *[redacted]
>> *Sent:* Thursday, January 16, 2020 4:33 PM
>> *To:*[redacted]
>> *Subject:* cells to isolate WH-CoV
>>
>> [redacted]
>>
>> Can you talk?
>>
>> I am currently in Japan. If you could let me know your phone number, I will call you.
>>
>> [redacted]
>>

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: hACE2 transgenic mice
Date: Wednesday, January 29, 2020 8:51:00 PM

Dear [redacted],

If you are going to do pathologic studies with hACE2 transgenic mice, I will not do the same experiment. I will do what others may not do; I do not think many people will do cat-to-cat and dog-to-dog transmission studies.

However, it would be great if you could provide me with a breeding pair of hACE2 transgenic mice because we want to use them for vaccine efficacy studies in my lab in Tokyo. Would that be possible?

Best,
[redacted]

From: [redacted]
Sent: Thursday, January 30, 2020 11:44 AM
To: [redacted]>
Cc: [redacted]
Subject: RE: hACE2 transgenic mice

H[redacted] sorry. We are planning on doing 2019-nCoV infections in the mice and performing pathologic evaluations. I obviously don't want to compete. How do you want me to proceed?

From: [redacted]
Sent: Wednesday, January 29, 2020 12:22 PM
To: [redacted]
Cc: [redacted]
Subject: hACE2 transgenic mice

Dear [redacted]

As I mentioned, I will be testing the growth of this virus in animals including marmosets, cats, dogs, ferrets, hamsters, and mice. I will also be examining vaccine candidates.

To this end, I am interested in obtaining your hACE2 transgenic mice. If you are going to examine the replication of 2019-nCoV in hACE2 transgenic mice and perform pathological analyses etc., we will not perform such studies.

Please let me know how we should proceed.
Thank you for your help!

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted] & coronavirus
Date: Friday, January 31, 2020 3:21:05 AM

Hi [redacted]

At present we believe our site visit will still go ahead, we have not been advised differently by [redacted].

I also imagine there will be some discussion on the impacts of the coronavirus within our main [redacted] call.

I will of course let you know if I receive any significant updates from [redacted] in the meantime.

[redacted]

[redacted]

On 29 Jan 2020, at 22:06, [redacted] wrote:

Does this mean our April visit will be cancelled too?
[redacted]

[redacted]
CC: [redacted]
[redacted] & coronavirus

Dear all,

I got a call from [redacted] this afternoon relating to the coronavirus and potential impacts upon our project. Some points from the call as follows (some more pertinent

to [redacted] but included for reference):

1. [redacted] staff are being switched to coronavirus related matters as a priority.

2. Therefore if we have any urgent matters for [redacted] we need to get them over ASAP. If you have any please let myself and [redacted] know.

3. This may further delay the [redacted] study data. In particular leadership are unlikely to be able to really review into the [redacted] study. A likely outcome is that [redacted] give us their take and then we further peer review the data in our own time, but perhaps not before the site meeting. In short [redacted] study remains a somewhat unknown quantity at present.

4. [redacted] asked that if we have any knowledge, involvement or anything related to coronavirus, could we share it in the spirit of collaboration. I believe [redacted] are reaching out to all their scientific partners in this manner. [redacted] do not have anything, but <u>if you could confirm for your labs that would be helpful thanks.</u>

5. [redacted] has been assigned to coronavirus analytics, there is a very small chance [redacted] may ask for some help with these analytics - to what extent and in what capacity remains unknown at present.

6. In general, all [redacted] staff will be less available than usual. I have indicated to [redacted] that of there is anything project related he wishes to push towards us we will be happy to help lighten their workload wherever possible.

7. I also updated [redacted] again that we are actively pursuing the budget and timelines for CVV production, with intention to get proposal to [redacted] at earliest possible opportunity.

Many thanks

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]
Date: Monday, February 17, 2020 3:25:23 PM

Thanks [redacted], thanks [redacted]

[redacted]

On Mon, Feb 17, 2020 at 9:03 PM [redacted] > wrote:

> [redacted],
>
> I agree with [redacted] explanation. However, if [redacted] cannot funnel money through [redacted], but can through [redacted], we could come up with an explanation. We could say that our approach provides broader coverage.
>
> Best,
>
> [redacted]
>
> **From:** [redacted]
> **Sent:** Tuesday, February 18, 2020 2:54 AM
> **To:** [redacted]
> **Cc:** [redacted] >
> **Subject:** CIVICs
>
> I emailed [redacted] on Friday re an informal talk re future funding prospects. Have not heard back yet.
>
> In preparing for that call, it occurs to me that he might ask about [redacted], and the possibility of funding though them. [redacted], can you help with that, I think you've explained before why that is unlikely, but would you say again please, and also any thoughts as to why our approach was not incorporated into a [redacted] proposal? [redacted] might ask, and I figure it is best I have something more than my current, I don't know.

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]
Date: Tuesday, February 4, 2020 5:36:36 PM

[redacted] is even better than [redacted], which is what I was thinking of for a name of just what I do here at [redacted] But, happy to share -- and it makes me feel like a real member of the Center to have contributed to the name. :-)

[redacted]

On Tue, Feb 4, 2020 at 6:28 PM [redacted] wrote:

> I like it!
> [redacted]
>
> > On Feb 4, 2020, at 6:12 PM, [redacted] > wrote:
> >
> > I like it too.
> >
> > Best,
> >
> > [redacted]
> >
> > ---
> >
> > **From:** [redacted] >
> > **Sent:** Tuesday, February 4, 2020 6:07:16 PM
> > **To:** [redacted]
> > **Cc:** [redacted]
> > **Subject:** RE: [redacted]
> >
> > Agree. [redacted] is good.

[redacted]

From: [redacted]
Sent: Wednesday, February 5, 2020 7:53 AM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]

Nice, I like it!

[redacted]

On 2/4/20 5:37 PM, [redacted] wrote:

> Sorry, my mistake writing the name. Yes, now has more sense.
>
> Thank you!
>
> ---
>
> **From:** [redacted]
> **Sent:** Tuesday, February 4, 2020 5:35:26 PM
> **To:** [redacted]
> **Cc:** [redacted]
> **Subject:** Re: [redacted]
>
> USE CAUTION: External Message.
>
> Makes more sense. Yes!

On Feb 4, 2020, at 17:34, wrote:

[External Sender]
Hi everyone,

Apologies the name is

So please, answer by Thursday, Feb 6:
"**YES**" if you **agree** with), and
"**NO**" if you **disagree**.

Thank you.

Kind regards,

From:
Sent: Tuesday, February 4, 2020 5:25:07 PM
To:
Subject:

Hi everyone,

Today we got an idea about the name of our research center (thanks We would like to change the name of " to "

We think [redacted] is more appropriated for the new [redacted] as we are not just working on pathogenesis, [redacted]

Please, answer by Thursday, Jan 6:
"**YES**" if you **agree** with [redacted] and
"**NO**" if you **disagree**.

Thank you!

Kind regards,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]

Subject: Re[redacted]
Date: Tuesday, February 4, 2020 4:50:58 PM

Yes [redacted]s good [redacted].

From: [redacted]
Sent: Tuesday, February 4, 2020 5:37 PM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]

Sorry, my mistake writing the name. Yes, now has more sense.

Thank you!

From: [redacted]
Sent: Tuesday, February 4, 2020 5:35:26 PM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]

USE CAUTION: External Message.

Makes more sense. Yes!

[redacted]

On Feb 4, 2020, at 17:34, [redacted] > wrote:

[External Sender]
Hi everyone,

Apologies the name is [redacted]

So please, answer by Thursday, Feb 6:
"**YES**" if you **agree** with [redacted] and
"**NO**" if you **disagree**.

Thank you.

Kind regards,

[redacted]

From: [redacted]
Sent: Tuesday, February 4, 2020 5:25:07 PM
To: [redacted]
Subject: [redacted]

Hi everyone,

Today we got an idea about the name of our research center (thanks [redacted] We would like to change the name of [redacted] to [redacted]. We think [redacted] is more appropriated for the new [redacted] as we are not just working on [redacted] as well.

Please, answer by Thursday, Jan 6:
"**YES**" if you **agree** with [redacted] and

“**NO**” if you **disagree**.

Thank you!

Kind regards,

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: cells to isolate WH-CoV
Date: Wednesday, January 22, 2020 8:08:37 AM

[redacted]

These will be vero cells.

[redacted]

From: [redacted]
Sent: Sunday, January 19, 2020 2:42 AM
To: [redacted]
Cc: [redacted]
Subject: Re: cells to isolate WH-CoV

Dear [redacted]

Thank you for your information.
I will make arrangement for shipping to World courier soon.

Could you tell me the name of cell line to fill in the invoice?
I check whether we need the document for clearance at import.

Best regards,
[redacted]
On 2020/01/18 1:12, [redacted] wrote:
> [redacted],
>
> You can contact me going forward regarding this shipment. Our shipping address is:
>
> [redacted]
>
> [redacted]
>
> I'm going to work on getting our export clearance which we'll need before we can ship. Thanks!
>
> [redacted]
>

> ----------
> *From:* [redacted]
> *Sent:* Friday, January 17, 2020 8:54 AM
> *To:* [redacted]
> *Cc:* [redacted]
> *Subject:* RE: cells to isolate WH-CoV
> Hi [redacted], I have cc'd the key people in my laboratory. They should be in touch shortly. [redacted]
>
> -----Original Message-----
> From: [redacted]
> Sent: Thursday, January 16, 2020 9:26 PM
> To: [redacted]
> Cc: [redacted]
> Subject: Re: cells to isolate WH-CoV
>
> Dear [redacted],
>
> I am [redacted] technical staff at [redacted] Tokyo lab and make arrangement of transfer of materials.
>
> I need your information to contact World courier.
> Could you let me know contact person and information for shipping?
>
> Best regards,
> [redacted]
>
> **********
> [redacted]
> **********
>
> On 2020/01/17 10:44, [redacted] wrote:
>> Dear [redacted]
>>
>> It was good to talk with you.
>>
>> I am writing here to introduce [redacted] who will coordinate getting a cell line to Japan

suitable for isolating the new coronavirus. She will work with World Courier to get the cells from your lab to Tokyo.
>>
>> I very much appreciate your help.
>>
>> If we are successful in isolating the virus, everyone can have access to it.
>>
>> [redacted]
>>
>> *From:* [redacted] >
>> *Sent:* Friday, January 17, 2020 9:06 AM
>> *To:* [redacted]
>> *Subject:* RE: cells to isolate WH-CoV
>>
>> Hi [redacted]. Anytime tonight [redacted]
>>
>> *From:* [redacted]
>> *Sent:* Thursday, January 16, 2020 4:33 PM
>> *To:* [redacted]
>> *Subject:* cells to isolate WH-CoV
>>
>> [redacted]
>>
>> Can you talk?
>>
>> I am currently in Japan. If you could let me know your phone number, I will call you.
>>
>> [redacted]
>>

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: hACE2 transgenic mice
Date: Tuesday, February 11, 2020 7:10:55 PM

[redacted]

I wanted to follow-up on when I should anticipate the MTA. Did I perhaps miss an email?

Thanks!
[redacted]

> On Jan 29, 2020, at 9:04 PM, [redacted] > wrote:
>
> I can route the paperwork when provided and have the assigned person connect with UNC. That way I can have control of the electronic record and have the ability to expedite. Would that work for everyone?
>
> Thanks
> [redacted]
>
> > On Jan 29, 2020, at 8:54 PM, [redacted] wrote:
> >
> > Thanks, [redacted]!
> >
> > [redacted] who would be the person?
> >
> > [redacted]
> >
> > ---
> >
> > **From:** [redacted]
> > **Sent:** Thursday, January 30, 2020 11:41 AM
> > **To:** [redacted]
> > **Cc:** [redacted]
> > **Subject:** RE: hACE2 transgenic mice
> >
> > Hi [redacted], We are going to need to set up a MTA agreement with Univ Wis. Madison for the transgenic hACE2 mice that are available in my laboratory. I will send you a blerb describing the mice shortly. [redacted], I will

need contact information for the people who deal with these things at UW-Madison. Talk with you soon. [redacted]

From: [redacted]
Sent: Wednesday, January 29, 2020 12:47 PM
To: [redacted]
Subject: RE: hACE2 transgenic mice

Thanks, [redacted]

From: [redacted]
Sent: Thursday, January 30, 2020 2:45 AM
To: [redacted]
Subject: RE: hACE2 transgenic mice

H[redacted]
[redacted] has no email today so he will be delayed in his response.
Warm regards,
[redacted]

From: [redacted]
Sent: Wednesday, January 29, 2020 12:22 PM
To: [redacted]
Cc: [redacted]>
Subject: hACE2 transgenic mice

Dear [redacted]

As I mentioned, I will be testing the growth of this virus in animals including marmosets, cats, dogs, ferrets, hamsters, and mice. I will also be examining vaccine candidates.

To this end, I am interested in obtaining your hACE2 transgenic mice. If you are going to examine the replication of 2019-nCoV in hACE2 transgenic mice and perform pathological analyses etc., we will not perform such studies.

Please let me know how we should proceed.
Thank you for your help!

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Today call
Date: Monday, February 3, 2020 5:07:00 AM

[redacted],

I am on my flight to Japan. I will be landing on the airport around the time when our call starts. So, I will be late in joining the call.

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: CVV generation
Date: Thursday, January 16, 2020 11:07:00 PM

Dear [redacted]

I am writing to see if you could help us with a project.
[redacted], and I are working under [redacted] support to establish [redacted]. In this project, we will need to make [redacted] vaccine strains under GMP conditions. Would it be possible to make them in your facility? Would you be able to test their pathogenicity in ferrets? I understand that ferret testing of candidate vaccines is required under WHO guidelines.

Best,
[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: FW: [redacted]
Date: Sunday, December 22, 2019 8:37:00 PM

Dear [redacted]

As far as we can tell from [redacted] emails, we will be contributing to the [redacted].
Regarding the [redacted]
Regarding the [redacted]
Please let us know what documents you'd need, and what your deadlines are.
[redacted] will write the proposals and can coordinate the details with you.

Thanks and Happy Holidays,

[redacted]

From: [redacted]>
Sent: Sunday, December 22, 2019 10:15 AM
To: [redacted]>
Subject: FW: [redacted]

From: [redacted]>
Sent: Sunday, December 22, 2019 4:55 PM
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]

[redacted]
Thanks.
Cell number is [redacted]
Let me know if you have a suggested title. Maybe we can have somewhat matching titles.
Something along the line of : [redacted] for me…
Cheers

[redacted]

[redacted]

CC: [redacted]

[redacted] Re: [redacted]

1) Cell number [redacted]
2) [redacted] I will provide with aims on [redacted] If there is a chance of cross-collaboration on both of these aims, please let me know as the scope/complexities of the projects could be expanded depending on how much collaboration we could have.

Happy Holidays!

[redacted]

On Dec 21, 2019, at 2:45 PM, [redacted] > wrote:

[External Sender]

Dear [redacted]

I finally had a scheme of how to put together the new [redacted] application, and your role on it.

First, budget for you: As discussed previously, I need to be very conservative with the budget to allow for everybody to be part of the new [redacted]. I think I told you I would like to allocate 200K for you in direct costs per year for seven years. I know is not too much, but hopefully you can manage with this, and then as options and pilots come available with the years, they will be opportunities to increase budgets, including collaborations with other [redacted]

As you know, the application asks for 4 components:; 1)Longitudinal human studies 2)Influenza surveillance, risk assessment and response research (4 possible projects) 3) Pandemic response and 4) Pathogenesis and immune response (minimal 3 projects)

You will be part of a [redacted] but for now I will need you to focus on [redacted]
One project is led by [redacted] on [redacted]. You will have [redacted]. [redacted] knows about it.
The other project is led by [redacted], on [redacted]. You will have [redacted] knows about it.

For the research components I will need from you:

1. Cell phone number we can reach you for any emergency. We promise we will only use it if really needed.
2. Contact [redacted] and [redacted] to tell them you will provide them with these aims
3. Send to [redacted] and [redacted] after coordinating with them your science components
4. Vertebrate animals (example included)
5. Select agent forms (example included)
6. Short CV. You are a main person in the grant, so I need a one page CV (see attached example from previous time)
7. Little blurb on your expertise in connection with [redacted] (see attached example from previous time)
8. Brief descriptions of other key personnel (see attached example from previous time)
9. Brief description of facilities and other resources (see attached example from previous time)
10. Collaboration letters (if pertinent).

Format (note this is different than R01s!!!):
a. Proposal page layout shall be letter size 8.5" x 11" for all pages.
b. Proposals shall not include links to internet web site addresses (URLs) or otherwise direct readers to alternate sources of information.

c. Proposals shall not include audio or video files of any type.
d. Font : Arial 11 points
e. Single spacing
f. Margins must be one-inch on all sides.
g. References. Do not format references, just include PMID numbers of references when you want to reference a paper. We will insert the references according to the PMID numbers.
h. Collaboration letters. Get them in word format, with letterhead and signatures inserted as pictures in the word format, in arial 10 points, single space. This is important as letters are part of the 250 pages limitation, so if we collect them this way, one letter will not be one page, but only half a page.

Deadlines:
For 1, 2: December 24
For 6, 7, 8 and 9: January 4
For 3: January 11 or whatever deadline is given to you by [redacted]
For 4, 5 and 10: January 18.

Compliance with this deadline will allow us to merge everybody and do several rounds of corrections and formatting.

[redacted], copied here, will send some other material we need from you required for the proposal (both technical and business), with also deadlines.

For administrative issues: Contact [redacted]
For anything else, including sending documents: Contact me and [redacted] the overall scientific manager of my lab who will help me in putting together the whole application. [redacted] will also take care of deadline compliance for the items requested in this email.

There will be a few more things needed, but for starters, this is all.

Let me know if OK with you and if you have any questions at this moment. [redacted] but I will try to be available

Happy Holidays and thanks for helping to put our new [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Alternative dates for [redacted] site visit
Date: Friday, January 10, 2020 4:14:00 AM

[redacted],

Options 1 and 3 work for me.

[redacted]

From: [redacted]
Sent: Friday, January 10, 2020 7:05 PM
To: [redacted]
Cc: [redacted]
Subject: Alternative dates for [redacted] site visit

Dear all,

[redacted] and I have been exploring alternative dates for our intended site visit.

We have identified the below dates which we think should work given the information we have received to date. We cannot have a site visit too near the beginning of March in case the [redacted] data does not come to us until end of Feb.

Could you please confirm that the options below would indeed work for you? We will then propose these as new alternatives to [redacted] et al.

Option 1.
- internal meeting on Thursday March 11th
- main site meeting with [redacted] on Friday March 12th

Option 2.
- internal meeting on Tuesday March 31st
- main site meeting with [redacted] on Wednesday April 1st

Option 3.
- internal meeting on Wednesday April 1st
- main site meeting with [redacted] on Thursday April 2nd

Many thanks

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted]
Date: Tuesday, December 24, 2019 6:55:00 AM

I will send an email to [redacted] to find out.

[redacted]

From: [redacted]
Sent: Tuesday, December 24, 2019 9:54 PM
To: [redacted] >
Cc: [redacted]
Subject: Re: [redacted]

Good morning/afternoon everybody,

[redacted] interpretation is what I also understood from [redacted] e-mail to me.

[redacted]

Since funding is an issue this time around, I trying to be as realistic as possible in regards to the type and number of animal studies that we could do. I do not have much in terms of leverage from other sources, but I remain optimistic about prospective grants that we are trying to put forward.

If there is anything I can help with, please do let me know.

I wish all Happy Holidays, Merry Christmas and look forward to working with you in 2020!

Cheers!

[redacted]

[redacted]

On Dec 24, 2019, at 4:10 AM, [redacted] wrote:

[External Sender]
Dear All,

As [redacted] stated, we seem to have received slightly different information from [redacted]

[redacted]

Happy Holidays,

[redacted]

From: [redacted]
Sent: Tuesday, December 24, 2019 1:09 AM
To: [redacted]
Cc: [redacted]
Subject: FW: CEIRR

[redacted]

[redacted] Good news is that I will get back on January 3, before the deadlines. I hope you can assist with getting this done on time.

[redacted]

Let me know what you think, so we can divide up the work.

Happy holidays

Van:
Datum: zaterdag 21 december 2019 om 20:23
Aan:
CC: >
Onderwerp: CEIRR

Dear

I finally had a scheme of how to put together the new CRIP application, and your role on it.

For the research component I will need from you:

1. Cell phone number we can reach you for any emergency. We promise we will only use it if really needed.
2. Title of your project (including component)
3. Project. A copy of the previous project is included as a reference for how it should be the formatted. Including component should not be more than 10 pages
4. Vertebrate animals (example included)
5. Select agent forms (example included)
6. Short CV. You are a main person in the grant, so I need a one page CV (see attached example from previous time)
7. Little blurb on your expertise in connection with CRIP(see attached example from previous time)
8. Brief descriptions of other key personnel (see attached example from previous time)
9. Brief description of facilities and other resources (see attached example from previous time)
10. Collaboration letters (if pertinent).

Format (note this is different than R01s!!!):
a. Proposal page layout shall be letter size 8.5" x 11" for all pages.
b. Proposals shall not include links to internet web site addresses (URLs) or otherwise direct readers to alternate sources of information.
c. Proposals shall not include audio or video files of any type.
d. Font : Arial 11 points
e. Single spacing
f. Margins must be one-inch on all sides.
g. References. Do not format references, just include PMID numbers of references when you want to reference a paper. We will insert the references according to the PMID numbers.
h. Collaboration letters. Get them in word format, with letterhead and signatures inserted as pictures in the word format, in arial 10 points, single space. This is important as letters are part of the 250 pages limitation, so if we collect them this way, one letter will not be one page, but only half a page.

Deadlines:
For 1, 2: December 28
For 6, 7, 8 and 9: January 4
For 3, 4, 5 and 10: January 18.

Compliance with this deadline will allow us to merge everybody and do several rounds of corrections and formatting.

copied here, will send some other material we need from you required for the proposal (both technical and business), with also deadlines.

For administrative issues: Contact
For anything else, including sending documents: Contact me and , the overall scientific manager of my lab who will help me in putting together the whole application. Marlene will also take care of deadline compliance for the items requested in this email.

There will be a few more things needed, but for starters, this is all.

Let me know if OK with you and if you have any questions at this moment. I will be traveling to Spain for Christmas, but I will try to be available

Happy Holidays and thanks for helping to put our new CRIP.

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted]
Date: Tuesday, December 24, 2019 7:41:00 AM

Dear [redacted]

Thank you for the clarification!

[redacted]

From [redacted]
Sent: Tuesday, December 24, 2019 10:36 PM
To: [redacted] >
Cc: [redacted]
Subject: RE: [redacted]

[redacted]

From: [redacted]
Sent: Tuesday, December 24, 2019 8:00 AM
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted]

USE CAUTION: External Message.

Dear [redacted]

Thanks for the information. We will try to plan accordingly.

[redacted]

Yours,

[redacted]

From: [redacted]
Sent: Tuesday, December 24, 2019 6:10 PM
To: [redacted]
Cc: [redacted]
Subject: RE [redacted]

Dear All,

As [redacted] stated, we seem to have received slightly different information from [redacted]

[redacted]

Happy Holidays,

[redacted]

From: [redacted]
Sent: Tuesday, December 24, 2019 1:09 AM
To: [redacted]

Cc: [redacted]
Subject: FW: CEIRR

[redacted]

[redacted] Good news is that I will get back on January 3, before the deadlines. I hope you can assist with getting this done on time.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Let me know what you think, so we can divide up the work.

Happy holidays
[redacted]

[redacted]
CC: [redacted]
[redacted]

Dea [redacted]

I finally had a scheme of how to put together the new CRIP application, and your role on it.

[redacted]

[redacted]

[redacted]

For the research component I will need from you:

1. Cell phone number we can reach you for any emergency. We promise we will only use it if really needed.
2. Title of your project (including [redacted] component)
3. Project. A copy of the previous project is included as a reference for how it should be the formatted. Including [redacted] component should not be more than 10 pages
4. Vertebrate animals (example included)
5. Select agent forms (example included)
6. Short CV. You are a main person in the grant, so I need a one page CV (see attached example from previous time)
7. Little blurb on your expertise in connection with CRIP(see attached example from previous time)
8. Brief descriptions of other key personnel (see attached example from previous time)
9. Brief description of facilities and other resources (see attached example from previous time)
10. Collaboration letters (if pertinent).

Format (note this is different than R01s!!!):
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h. Collaboration letters. Get them in word format, with letterhead and signatures inserted as pictures in the word format, in arial 10 points, single space. This is important as letters are part of the 250 pages limitation, so if we collect them this way, one letter will not be one page, but only half a page.

Deadlines:
For 1, 2: December 28
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Compliance with this deadline will allow us to merge everybody and do several rounds of corrections and formatting.

[redacted] copied here, will send some other material we need from you required for the proposal (both technical and business), with also deadlines.

For administrative issues: Contact [redacted]
For anything else, including sending documents: Contact me and [redacted] the overall scientific manager of my lab who will help me in putting together the whole application. [redacted] will also take care of deadline compliance for the items requested in this email.

There will be a few more things needed, but for starters, this is all.

Let me know if OK with you and if you have any questions at this moment. [redacted], but I will try to be available

Happy Holidays and thanks for helping to put our new [redacted]

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: [redacted]
Date: Tuesday, January 7, 2020 6:01:48 AM

Dear [redacted] and [redacted] team,

Thanks a lot for the draft. We will integrate our research into your proposal.
We will have it ready by the end of Jan 12th.

All the best,

[redacted]

From: [redacted]
Sent: Tuesday, January 7, 2020 5:40 AM
To: [redacted] >
Cc: [redacted] >
Subject: [redacted]

Dear [redacted]

Sorry for the delay in communication during my holidays.

Based on your inputs so far, and internal discussions, we have come to a proposal for a set of Objectives and Specific aims for our part of the research proposal, as indicated below and in the attached doc. It includes [redacted] suggestion to have [redacted] and [redacted] aims on H9. If [redacted] prefers, his aims (now under Objective 4) can be easily split over Objectives 1-3, to make a more comprehensive/integrated package; I leave that up to [redacted] Please all have a quick look and let me know if you prefer alterations.

[redacted] wants us to stick to the old format of the research proposal (attached, with new section A, and sections B-G in gray text from the previous proposal). Now that we have a draft of section A (Objectives and aims) (0.5 page) I suggest we move on with section D (Preliminary results, approach, and methods, organized per Objective and Aim), asking for your detailed input by Sunday night, January 12. That would allow us all to look at each other's aims, propose collaborations/interactions where it fits and collectively write the other sections B (Background and Significance, 1.5 page), C (Summary from previous accomplishments in [redacted], <0.5 page), E (Interactions with other projects, <0.5 page), F (Schedule, <0.5 page) and G (References, 1 page). In section D, we have roughly 5.5 to 6 pages. This means we only have 1.5 pages per Objective, or 2-3 aims per page. We thus need to be VERY brief about preliminary data, approach and methods, including potential nice display (prelim data) items. Let me know if you have problems with this proposal and the timelines. Of course, feel free to start providing input for sections B, C, E, F, G as well if that is easier for you. But it would be

nice to have the aims worked out in a week, to than have another week for finalization. Agreed?

Kind regards,
[redacted]

Proposed new section A (also already edited in the attached doc):
Project 4: [redacted]

From: [redacted]
To: [redacted]
Subject: RE: First draft project [redacted]
Date: Wednesday, January 15, 2020 6:27:01 AM
Attachments: image001.png
image002.png
image003.png

Ok, good point. I will simply refer to your RP and inform [redacted] of the issue of PIs on multiple projects.
[redacted]

Yours sincerely,

[redacted]

[redacted]

[redacted] RE: First draft project [redacted]

Dear All,

As [redacted] stated, [redacted] and [redacted] accomplishments are described in 'our' RP on host factors.
[redacted], you could just refer to our RP.
[redacted] should consolidate this for investigators who are on several projects.

Thanks,

[redacted]

From: [redacted]
Sent: Wednesday, January 15, 2020 5:34 AM
To: [redacted]
Cc: [redacted]
Subject: Re: First draft project [redacted]

Good morning everybody,

As I see a section on accomplishments on [redacted] proposal and also one in [redacted]. Since at least in my case it will list repetitive information, I wonder if [redacted] would like to consolidate those into a single section somewhere else in the proposal. I think it should be a section at the beginning of the main proposal.

On Jan 15, 2020, at 2:46 AM, wrote:

OK,

From:
Sent: Wednesday, January 15, 2020 4:28 PM
To:
Cc:
Subject: Re: First draft project

Hi
Yes it was received and your comments and those of have been implemented (attached). I don't think there is space for additional display items, as we already reach the limit but I will see again when we are done.
Please remember that I still expect from you and a few sentences on your most important previous accomplishments in 2 or 3 sentences should be enough in section C.
Thanks

PS you indicated that the structuring of objectives/aims should be different, but this is what we did 7 years ago, and how instructed me to do it again.....

[redacted]

CC: [redacted]

[redacted]Re: First draft project [redacted]

Could you guys let me know if you saw the e-mail below? (I have removed the attachments here, just in case it was a cause for trouble).

Bests!

[redacted]

[redacted]

On Jan 13, 2020, at 4:39 PM, [redacted] wrote:

Hi All,

Minor changes, mostly addressing [redacted] comments. Please also find attached a slide that shows preliminary results of our aerosol system. I think it could complement very well what you are doing. In the slide, you find a virus that is [redacted] we are generating data on a non transmissible but it will take some time.

[redacted]

<20191031_[redacted]1.pptx>

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: First draft project [redacted]
Date: Monday, January 13, 2020 3:04:00 PM
Attachments: [redacted] Proposal V1yk [redacted] .docx

Dear All,

Attached please find a few minor edits/comments (added to the document [redacted] sent earlier).
If I understand [redacted] correctly, we don't need a reference list.
Throughout, there are sections that could be condensed (I pointed out a few, but there are others).

Overall, it reads very well!

Thanks,

[redacted]

From: [redacted]
Sent: Monday, January 13, 2020 10:12 AM
To: [redacted]
Cc: [redacted]
Subject: RE: First draft project [redacted]

[redacted]

Thank you for putting this together.
Please see attached my comments; they are all minor.
To make it short, we could reduce the size of some of the figures.

Best,
[redacted]

From: [redacted]
Sent: Monday, January 13, 2020 9:44 PM

To: [redacted]
[redacted]>
Cc: [redacted]
Subject: First draft project [redacted]

Hi [redacted],
Thank you very much for your input so far. We have pulled things together in a first draft proposal, attached. I hope we treated your pieces of text satisfactory. Please do not hesitate to correct us if we did not. This is a good time to go through the proposal and make corrections (with track changes). Although we will also continue to make improvements, time is short so we have to work in parallel. As a minimum, please check if we inserted your aims (section A) and proposed work (section D) correctly. In addition, we now need your input in sections C and E. [redacted] needs to check if we filled out schedule F correctly, and [redacted] needs to provide this info as well.
Please note that we are now at 12 pages, 2 pages over the limit that [redacted] gave us. So feel free to go through the text and make suggestions to shorten it. And whatever you add: be brief!
Apologies if we forgot to insert information that was important to you. If you feel strongly about it, now is the time to add it back!
Please provide us with feedback in the next few days. Any suggestions are welcome…
Cheers
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: First draft project [redacted]
Date: Monday, January 13, 2020 10:10:00 AM
Attachments: [redacted] V1yk.docx

[redacted],

Thank you for putting this together.
Please see attached my comments; they are all minor.
To make it short, we could reduce the size of some of the figures.

Best,
[redacted]

From: [redacted]
Sent: Monday, January 13, 2020 9:44 PM
To: [redacted]
Cc: [redacted]
Subject: First draft project [redacted]

Hi [redacted]
Thank you very much for your input so far. We have pulled things together in a first draft proposal, attached. I hope we treated your pieces of text satisfactory. Please do not hesitate to correct us if we did not. This is a good time to go through the proposal and make corrections (with track changes). Although we will also continue to make improvements, time is short so we have to work in parallel. As a minimum, please check if we inserted your aims (section A) and proposed work (section D) correctly. In addition, we now need your input in sections C and E. [redacted] needs to check if we filled out schedule F correctly, and [redacted] needs to provide this info as well.
Please note that we are now at 12 pages, 2 pages over the limit that [redacted] gave us. So feel free to go through the text and make suggestions to shorten it. And whatever you add: be brief!
Apologies if we forgot to insert information that was important to you. If you feel strongly about it, now is the time to add it back!
Please provide us with feedback in the next few days. Any suggestions are welcome...
Cheers
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: RE: Using [redacted]
Date: Wednesday, January 15, 2020 3:49:00 PM
Attachments: image001.png
image002.png
image003.png

If the [redacted] free, that is the way to go. They will use their own [redacted]

[redacted]

From: [redacted]
Sent: Wednesday, January 15, 2020 10:45 PM
To: [redacted]
Cc: [redacted]
Subject: RE: Using [redacted]

> Did this not occur with [redacted] so that this would be not a problem when [redacted] backbone would be used?
>
> Yours sincerely,
>
> [redacted]

[redacted]

[redacted]
CC: [redacted]
[redacted] Using [redacted]

All

[redacted] and I are having a call with [redacted] and [redacted] tomorrow regarding funding for the [redacted] generation.

One of the things [redacted] has mentioned to [redacted] is to say again, as he did to all of us, that [redacted] might make the CVVs for free.

We'd previously decided to not ask [redacted] because of their [redacted] I think it was) with a [redacted] they [redacted] that could not be replicated in your lab.

I wonder how to say this, or whether it is OK to say this at all, to [redacted] and [redacted] --as clearly a non-cost [redacted]

would be attractive to them.

What are your thoughts?

From: [redacted]
To: [redacted]
Subject: RE: funding strategy call
Date: Friday, December 6, 2019 5:57:00 AM

Works for me.

[redacted]

From: [redacted]
Sent: Friday, December 6, 2019 8:37 PM
To: [redacted]
Subject: Re: funding strategy call

Thanks everyone. It looks like next Wednesday 11th would be the preferred date. How about:

6am [redacted]
12pm [redacted]
1pm [redacted]
9pm [redacted]

If that's ok with everyone we will go ahead and send out the Webex invite.

Thanks,

[redacted]

On 5 Dec 2019, at 19:31, [redacted] wrote:

Wednesday works for me. Tuesday not.

From: [redacted]
Sent: Thursday, December 5, 2019 8:06:00 PM
To: [redacted]
Cc: [redacted]
Subject: RE: funding strategy call

I am available in the evening of Dec 10 (Tue) and 11 (Wed).

[redacted]

From: [redacted]
Sent: Friday, December 6, 2019 2:29 AM
To: [redacted]

Cc: [redacted]

Subject: RE: funding strategy call

With [redacted] being in Japan next week, I'd suggest an early morning time in the US / early afternoon in Europe / evening in Japan (if [redacted] is available).
I can be flexible on Tue and Wed.

[redacted]

From: [redacted]
Sent: Thursday, December 5, 2019 11:20 AM
To: [redacted]
Cc: [redacted]
Subject: funding strategy call

All

Perhaps it would be useful to have a funding strategy call now we have more of a picture of how [redacted] will look. FYI [redacted] proposed budged is reduced to 1/3d, from $300k/year to $100k/year.

I'm about to go on vacation until monday evening. So suggest either Tuesday morning [redacted] time, or anytime on Wednesday next week. I'd prefer Wednesday.

[redacted] would you see if there is a suitable time among the replies please and set a time (assuming people agree to a call).

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: Alternative dates for [redacted] site visit
Date: Friday, January 10, 2020 1:45:31 PM

Option 2 and 3 only work for me.
[redacted]

From: [redacted]
Date: Friday, 10 January 2020 at 12:24
To: "[redacted]
Cc: "[redacted]
Subject: RE: Alternative dates for [redacted] site visit

[redacted]

For me, all three options work.

[redacted]

From: [redacted]
Sent: Friday, January 10, 2020 4:16 AM
To: [redacted] >
Cc: [redacted]
Subject: RE: Alternative dates for [redacted] site visit

[redacted]

Options 1 and 3 work for me.

[redacted]

From: [redacted]
Sent: Friday, January 10, 2020 7:05 PM
To: [redacted]
Cc: [redacted]

Subject: Alternative dates for [redacted] site visit

Dear all,

[redacted] and I have been exploring alternative dates for our intended site visit.

We have identified the below dates which we think should work given the information we have received to date. We cannot have a site visit too near the beginning of March in case the [redacted] data does not come to us until end of Feb.

Could you please confirm that the options below would indeed work for you? We will then propose these as new alternatives to [redacted] et al.

Option 1.
- internal meeting on Thursday March 11th
- main site meeting with [redacted] on Friday March 12th

Option 2.
- internal meeting on Tuesday March 31st
- main site meeting with [redacted] on Wednesday April 1st

Option 3.
- internal meeting on Wednesday April 1st
- main site meeting with [redacted] on Thursday April 2nd

Many thanks

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: An extra couple of topics for our monthly call on Thursday
Date: Monday, January 13, 2020 11:02:53 AM

---

yes a shame

[redacted]

On Mon, Jan 13, 2020 at 4:09 PM [redacted] > wrote:

> Thanks, [redacted]
>
> [redacted]
>
> ---
>
> **From:** [redacted]
> **Sent:** Tuesday, January 14, 2020 1:08 AM
> **To:** [redacted]
> **Cc:** [redacted]
> **Subject:** Re: An extra couple of topics for our monthly call on Thursday
>
> Ah, yes. Still a shame. Lobbying work for you (or [redacted] to be done....
> [redacted]
>
> ---
>
> [redacted] RE: An extra couple of topics for our monthly call on Thursday
>
> [redacted]

My understand is that we can continue to use the high yield PR8 backbone for preclinical work. For the CVV generation that has to be done under GMP/GLP conditions, [redacted] (Japan) have to use "their" PR8 backbones, which I think are not identical.

Best,

[redacted]

From: [redacted]
Sent: Tuesday, January 14, 2020 12:23 AM
To: [redacted]
Cc: [redacted]
Subject: Re: An extra couple of topics for our monthly call on Thursday

Ugh. We need to remake the CVVs with another PR8 backbone? Which one?
[redacted]

[redacted]

CC: [redacted] RE: An extra couple of topics for our monthly call on Thursday

Hi,

Following our discussion on the monthly teleconference on Jan 9, 2020 and follow-up discussion with leadership.

> 1. Whether we should, as per our previous discussions, go ahead with an [redacted] You had

> previously concurred with us that this would be valuable primarily because of all recent zoonoses

> being [redacted], and because no clinical study with [redacted] has yet been performed to our knowledge. Your risk

> assessment we think had determined [redacted] A decision on this soon will allow us to

> work up our [redacted] for use in ferret challenge pre-clinical work,

> stability testing, and CVV generation.

An [redacted] is acceptable within the context of this contract as previously discussed (Aug 14, 2019 email) and from a programmatic perspective. We will rely on your expertise on whether to pursue the [redacted] work given the risk.

From our understanding [redacted] will be exactly the same for all CVV – what is the status/timeline for [redacted]?

Remember to submit a contracting officer authorization request for the CVV generation work given the remaining time and budget on the contract. Please prioritize the CVV. Note, the [redacted] work reference here will not bridge over to the [redacted]).

> 2. Discussion on CVV generation. In particular:

- whether you would also [redacted]: Yes
- whether we should [redacted]: Egg
- whether we should be doing our [redacted]:

Refer to the guidance

- whether we can use the [redacted]: NO
- is using a recombinant HA vaccine still on the table? Yes, but at this point it is a difficult implementation path

Let us know if you have any further questions.

Regards,

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

From: [redacted]
Sent: Tuesday, January 7, 2020 10:18 AM
To: [redacted]>
Cc: [redacted]
Subject: Re: An extra couple of topics for our monthly call on Thursday

Thanks [redacted]

[redacted]

On Tue, Jan 7, 2020 at 2:38 PM [redacted] wrote:

Hi,

Ok. We should leave the last 10-15 minutes for this discussion.

Wendell: please join us in [redacted] at 9:45am [redacted] time Thursday, Jan 9, 2020.

Regards,

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

From: [redacted]
Sent: Monday, January 6, 2020 4:47 PM
To: [redacted] >
Cc: [redacted]
Subject: An extra couple of topics for our monthly call on Thursday

Dear [redacted] and [redacted]

In addition to our normal reporting during our monthly call with you this Thursday. We would find it useful to have a discussion on the following please.

1. Whether we should, as per our previous discussions, go ahead with an [redacted]. You had previously concurred with us that this would be valuable primarily because of all recent zoonoses being [redacted], and because no clinical study with [redacted] has yet been performed to our knowledge. Your risk assessment we think had determined [redacted]. A decision on this soon will allow us to work up our [redacted] for use in ferret challenge pre-clinical work, stability testing, and CVV generation.

2. Discussion on CVV generation. In particular:
   - whether you would also like [redacted]
   - whether we should [redacted]
   - whether we should be doing our [redacted]
   - whether we can use the [redacted]
   - is using a recombinant HA vaccine still on the table?

Best regards
[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]
Date: Friday, January 10, 2020 5:48:57 AM

Hi [redacted]
I think I can integrate your map picture if you make it small (visible as max 2x2 inch). I will make a composite figure with yours as one of the panels. Your text description refers to the colors in that picture, so I think we need it.
Rest is good!
[redacted]

[redacted]
CC: [redacted]
[redacted] Re: [redacted]

Hi Everybody,

Please find attached the 3 pages version of the [redacted] roposal. I would love to see it embedded in objectives 1-3. I have cut as much as I could as well as reduced the number of references from an earlier version that I shared with [redacted] this morning (following [redacted] advice). I was not able to include any figure without adding more pages. If there is a chance that I could include 1 figure on the cartography later on when you have a better notion of the entire proposal, please let me know.

[redacted], I am also attaching the 3 pages version of the [redacted] proposal, down from the almost 6 that I had shared with you before. In this new version, I have tighten as much as I could and reduced the number of references as well.

In both of these documents I have added the PMIDs at the end of the document. Not sure if you guys use Endnote, but I am attaching two libraries with the references for each proposal.

If you guys think the proposals are worth it and would like to collaborate with us, please let me know.

Many Thanks!

I'll await further instructions.

Cheers!

[redacted]

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: Draft for [redacted]
Date: Monday, January 6, 2020 3:51:41 PM

---

thanks both. i've sent to [redacted] you should have been copied.

noted [redacted], re your travel on thursday

[redacted]

On Mon, Jan 6, 2020 at 9:04 PM [redacted] > wrote:

> Looks good to me too.
>
> In relation with point 2 (the desire to make a cvv) it would be good to know if recHA protein is off the radar as an option for [redacted] If it is not, than point 1 could become less relevant if we decide to go with recHA. If it is, than [redacted] may not wish to continue proposed work with recHA that was started previously (we made the wildtype and [redacted]
>
> ---
>
> [redacted]
> **CC:** [redacted]
> [redacted] RE: Draft for [redacted]
>
> [redacted]
>
> I have nothing to add/change.
>
> I will be arriving at O'Hare at 7:05am on Jan 9th.
>
> So, I may miss the beginning of this [redacted] call. But, I will join the call as soon as I get through immigration.
>
> [redacted]

From: [redacted]>
Sent: Tuesday, January 7, 2020 5:32 AM
To: [redacted]>
Cc: [redacted]
Subject: Draft for [redacted]

[redacted]

Draft below for [redacted] as per our discussion earlier today. Any changes?

[redacted]

Dear All

In addition to our normal reporting during our monthly call with you this Thursday. We would find it useful to have a discussion on the following please:

1. Whether we should, as per our previous discussions, go ahead with an [redacted]. You had previously concurred with us that this would be valuable primarily because of all recent zoonoses being [redacted], and because no clinical study with [redacted] has yet been performed to our knowledge. Your risk assessment we think had determined [redacted] A decision on this soon will allow us to work up our [redacted] for use in ferret challenge pre-clinical work, stability testing, and CVV generation.

2. Discussion on CVV generation. In particular:

- whether you would also like [redacted]
- whether we should [redacted]
- whether we should be doing our [redacted]

- whether we can use the [redacted]

Best regards

[redacted]

From: [redacted]
To: [redacted]
Cc: [redacted]
Subject: Re: [redacted]
Date: Tuesday, January 14, 2020 9:46:01 AM

Thanks [redacted] for your quick reply.

When [redacted] did not reply, I went back and realized I'd mistakenly not sent the email to her. She can do Friday also. So let's make it [redacted] please, this Friday. We'll send a webex.

[redacted] also replied: "And we have indeed infected ferrets last week with [redacted]

[redacted]

On Mon, Jan 13, 2020 at 8:07 PM [redacted] > wrote:

> I have another meeting Thursday at 2. The Friday timeslot is OK.
> [redacted]
>
> ---
>
> [redacted]
> **CC:** [redacted]
> [redacted] RE: [redacted]
>
> Dear All,
>
> With [redacted] being in Japan on Thu/Fri, we could talk as follows:
>
> - Thu: [redacted]
> - Fri: Between [redacted]
>
> Best,

[redacted]

From: [redacted]
Sent: Tuesday, January 14, 2020 2:39 AM
To: [redacted] >
Cc: [redacted]
Subject: Re: [redacted]

If you could also give what times are possible on Thursday this week too please, that would be good, in case we are ready by then, as [redacted] is on vacation on Friday.

Similarly [redacted], what times can work for you on Thursday and Friday this week?

I expect we might need 45 to 60 minutes for the call.

[redacted]

On Mon, Jan 13, 2020 at 5:36 PM [redacted] wrote:

> [redacted]
>
> cc [redacted]
>
> [redacted] and I talked on Friday re coming up with a priority order for testing their [redacted] viruses with an [redacted]

[redacted] is pulling together the data they have for the [redacted] parents. [redacted] are doing some [redacted].

We expect to be ready by Thursday or Friday for a call to discuss. Would you be available to join a call on Friday, and if so what times?

Also, do I remember correctly that you were going to try infecting ferrets with [redacted] to see if it is immunogenicity enhanced in your hands?

[redacted]